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Breastfeeding and infant neurodevelopment in a cohort with sibling pair analysis: the Japan Environment and Children's Study

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ABSTRACT

Objectives

To investigate the association between breastfeeding and infant neurodevelopment during the first year of life using sibling comparison.

Design

Nationwide prospective birth cohort study with sibling pair analysis.

Setting

15 regional centres that participated in the Japan Environment and Children's Study.

Participants

This study included 77 119 children (singleton, term birth and no malformation/severe diseases) whose mothers were registered between January 2011 and March 2014, including 3 521 duos or trios of siblings.

Primary outcome measures

The primary outcome was neurodevelopmental delay at 6 and 12 months of age, assessed using the Japanese translation of the Ages and Stages Questionnaires, third edition. Logistic regression analyses adjusted for confounders were performed to estimate the odds ratios of delay associated with dichotomous statuses of any or exclusive breastfeeding. Pairs of siblings discordant for statuses were selected, and conditional logistic regression analyses were conducted with a matched cohort design.

Results

Neurodevelopmental delay was identified in 6 162 (8.4%) and 10 442 (14.6%) children at 6 and 12 months of age, respectively. Any breastfeeding continued during the first 6 months and first 12 months after birth was associated with reduced neurodevelopmental delay at 12 months of age (adjusted odds ratio [95% confidence interval]: 0.80 [0.75 to 0.85] and 0.80 [0.76 to 0.84], respectively). Furthermore, exclusive breastfeeding during the first 3 months was associated with reduced neurodevelopmental delay at 12 months of age (0.84 [0.80 to 0.88]). In sibling pair analysis, the association between any breastfeeding during the first 12 months and reduced neurodevelopmental delay at 12 months of age persisted (0.59 [0.39 to 0.91]).

Conclusions

The present study demonstrated for the first time the association of continuous breastfeeding with reduced neurodevelopmental delay at 1 year of age using sibling pair analysis. This less-confounded association provides an argument to promote breastfeeding continuation.

Strengths and limitations of this study

- This study for the first time demonstrated the association between breastfeeding and neurodevelopment using sibling comparison, which strongly controls for sibling-shared factors.
- Our results provide an argument to promote continued breastfeeding during the first year of life.
- Monitoring of the ongoing cohort of the JECS will reveal the later effects of breastfeeding.

INTRODUCTION

Since 1929, the beneficial effects of breastfeeding on brain development have been repeatedly demonstrated.[1-4] Many observational studies[5-8] demonstrate that breastfeeding is associated with better cognitive outcomes, including neurodevelopment, language, and intelligence. However, this association can be produced by differences in demographic, socioeconomic, and environmental factors between mothers who breastfeed and those who do not.[9-12] In high-income countries, mothers with higher levels of education, social position, income, and intelligence are more inclined to breastfeed and to do so more exclusively and for a longer duration. Thus, their children are more likely to have higher cognitive functions, which can result in a superficial association between breastfeeding and better child cognition. In previous studies, the association disappeared or became highly diminished after controlling for confounders, especially maternal intelligence.[9,13,14] Nonetheless, a recent meta-analysis concluded that breastfeeding was significantly associated with higher cognitive abilities, even after adjusting for such confounding factors.[3]

After explicitly controlling for these measured factors, unmeasured—even unknown—confounders such as parental characteristics and child-rearing practices remained. To further control for these confounders, previous studies[9,15-17] conducted sibling pair analysis in investigating the association of breastfeeding with child cognitive outcomes. These analyses focused on siblings pairs who were discordant for breastfeeding exposure. A sibling pair from the same mother largely shares parental and environmental factors. Thus, the effects of these confounders can be cancelled out when the pair is matched in the analysis. However, on this topic, sibling pair analysis is challenging because little variation in breastfeeding often exists between siblings, which may reduce statistical power and erroneously cause null findings.[17] To our knowledge, only three studies[9,15,16] have examined the association between breastfeeding and cognitive functions using this method, and these studies all produced statistically null effects. The reason for the null results remains unclear. However, these findings may be accounted for by the study designs: data on feeding status were collected only once within 1 year[9] or 2 years[15] after a child's birth or in adolescence.[16]

The goal of the current study was to investigate the association between breastfeeding and child neurodevelopment during the first year of life by using data from the Japan Environment and Children's Study (JECS). This nationwide birth cohort study includes >100,000 children and thus enables sibling pair analysis with a sufficient number of participants. The monthly status of breastfeeding was collected repeatedly in the first year of life, thereby minimizing the risk of recall bias. The beneficial effects of breastfeeding on cognitive development decrease as children age;[18] therefore, investigating the association between breastfeeding and cognitive development during early childhood has the advantage of allowing researchers to infer the role of breastfeeding on the developing brain.

METHODS

Design

The JECS is a nationwide, multicenter, prospective birth cohort study funded by the Ministry of Environment of Japan. The details of the study design have been described elsewhere.[19,20]

Briefly, pregnant participants were registered between January 2011 and March 2014 in 15 regional centers covering a wide geographical area in Japan. During pregnancy, data on demographics, smoking, education, and socioeconomic statuses were obtained during the first and second/third trimesters by using self-administered questionnaires. Detailed information regarding the mother and child was obtained from medical records transcripts during the first trimester, at the time of delivery, and when the child was 1 month old. After delivery, data on feeding style, use of complementary foods, neurodevelopmental status, and affected diseases were collected at ages 1 and 6 months and every 6 months until the child was 6 years old, and then twice a year thereafter *via* self-reported questionnaires completed by the parents.

The JECS protocol was reviewed and approved by the Ministry of Environment's Institutional Review Board for Epidemiological Studies (No.100910001) and by the ethics committees of all participating institutions (No.2019-070). The ethical approval for this study was an extension of the ethical approval for the JECS protocol. Written informed consent was obtained from all parents.

Participants

In this study, we used the fixed dataset "jecs-an-20180131" that was released in March 2018. This dataset contains all available data extracted from the aforementioned questionnaires and records until a child was 12 months old. The data for 104 065 fetuses from 103 062 pregnancies were linked to the respective maternal data. The participants selected were 92 381 live-born singleton children, delivered at term (≥37 gestational weeks and <42 gestational weeks), of parents of Japanese nationality and for whom information on sex and birthweight had been recorded (Figure 1). Of these children, those who had malformations or severe diseases, or who had missing information on feeding style during the first year of life or neurodevelopment at 6 months and 12 months old were excluded. After these exclusions, the data of 77 119 children were included in our analysis.

Exposure

The main exposure factor was breastfeeding. Mothers were asked to fill in the monthly feeding status of their child by using questionnaires when the child was 1 month, 6 months, and 12 months old. This information included whether the child was breastfed, formula-fed, or both. The questionnaire administered when the child was 12 months old also queried when complementary food was first started. Breastfeeding duration was the duration for which the child was breastfed, irrespective of concurrent consumption of formula milk. We also dichotomously assessed whether or not a child continued (1) any breastfeeding during the first 6 months, (2) any breastfeeding during the first 12 months, (3) exclusive breastfeeding during the first 3 months, and (4) exclusive breastfeeding during the first 6 months. Breastfeeding was "exclusive" if the child consumed only breastmilk—and nothing else (no consumption of formula milk or complementary foods) during these periods.

For sibling pair analysis, we selected pairs who were discordant on the status of any breastfeeding or exclusive breastfeeding. When discordance was observed among three siblings (e.g., only one of the three children was breastfed), we randomly selected one of the two siblings who were not breastfed and then paired the selected one with the breastfed sibling.

Outcome

The outcome was neurodevelopmental delay measured at 6 months and 12 months old, using the Japanese translated version of the Ages and Stages Questionnaires: A Parent-Completed Monitoring System, third edition (ASQ-3). This version was prepared through a back-translation procedure and was approved by the publisher of the original English version.[21] The ASQ-3 can identify infants or young children who need further neurodevelopmental assessment to determine whether they are eligible for early intervention. The findings of the questionnaire basically agree with those of professionally administered developmental batteries.[22,23] It has been used in clinical and research settings and translated into several languages.[24-27] The ASQ-3 assesses five developmental domains. For each domain, six skills are described to which parents answer “yes,” “sometimes,” or “not yet,” depending on whether their child is demonstrating the described skill. The responses are converted to points, with “yes” receiving 10 points; “sometimes”, 5 points; and “not yet”, 0 points. The child’s score for each developmental domain is the sum of all points received for the items under that domain and ranges from 0 to 60 points. The cut-off score for each domain was defined as two standard deviations below the mean score of large standardized samples in the United States of America. A child was defined as having a neurodevelopmental delay if a score was at or below the cut-off level in any developmental domain. When the cut-off scores of the original English version were used in our population, an excessive number of children were classified as having a developmental delay (47.4% and 34.6% for 6 months and 12 months, respectively). Although preliminary cut-off scores of the Japanese translation were recently proposed,[28] these were not recommended to be used with confidence before 24 months old because of very limited sample sizes. Therefore, the cut-off scores were determined by using the same methodologies used in the original version, based on available data at ages 6 months (n = 82 410) and 12 months (n = 78 442) (Figure 1), which would represent the general Japanese population.

Statistical analysis

To assess the association of breastfeeding with child neurodevelopment, we conducted logistic regression analyses adjusted for the following covariates: i) sex, ii) gestational age, iii) birthweight, iv) mother's age, v) maternal smoking status during pregnancy, as recorded in the first trimester, vi) maternal and vii) paternal education level (junior high school, high school, and university or graduate school), viii) annual family income (<4 000 000; 4 000 000–5 999 999; ≥6 000 000 JPY), ix) introduction of complementary foods before 6 months old, x) home speech stimulation at 1 month (whether a mother did or did not talk to her baby habitually), and (xi) home speech stimulation at 12 months (whether a mother read picture books and then talked to the child three times or more weekly *vs.* fewer than 3 times). The "home speech stimulation" covariate at the two age points were used instead of the Home Observation for Measurement of the Environment scale,[29] which is not employed in the JECS.

For sibling pair analysis, we conducted conditional logistic regression analyses with 1:1

matched cohort data of sibling pairs whose breastfeeding statuses were discordant.[30] The adjusted covariates were as follows: i) sex, ii) gestational age, iii) birthweight, iv) order of siblings in the discordant pair, v) maternal smoking status during either pregnancy, vi) complementary food introduction, vii) home speech stimulation at 1 month old, and viii) home speech stimulation at 12 months old. All statistical analyses were conducted using R software (version 3.5.0). Conditional logistic regression analyses were conducted using "survival" (version 2.41.3) in the R package. We reported crude and adjusted odds ratios (aORs) with 95% confidence intervals (CIs). The level of significance was $P = 0.05$.

Patient and public involvement

No participants were involved in creating the research question or the outcome measures, nor were they involved in developing plans for recruitment, design or implementation of the study. No participants were asked to provide advice on the interpretation or writing up of the results. There are plans to disseminate the results of the research to study participants and the general public. Participants were thanked in the acknowledgments.

RESULTS

The baseline characteristics of 77 119 children are summarized in Table 1. Nearly all (76 167, 98.8%) children were started on any breastmilk during their first month of life. Any breastfeeding was continued during the first 6 months and 12 months of life in 82.1% and 64.4% of children, respectively. Exclusive breastfeeding was continued during the first 3 months and 6 months of life in 39.6% and 20.3% of children, respectively. Neurodevelopmental delay was identified in 8.4% and 14.6% of children at 6 months and 12 months old, respectively. The sibling cohort included 3521 sibling sets (7055 children) in total: 3508 duos (7016 children) and 13 trios (39 children). The characteristics of the sibling sample were substantially similar to those of the full sample. Nevertheless, the sibling sample appeared to have weak tendencies towards younger maternal age, lower paternal education, lower family income, lower rates for any breastfeeding during the first 12 months old, and higher rates for exclusive breastfeeding during the first 3 months.

For the full sample (n = 77 119), we conducted logistic regression analyses, while adjusting for confounders, to examine neurodevelopmental delay in relation to various types of breastfeeding exposures. Shorter durations of any breastfeeding were associated with a higher risk of neurodevelopmental delay at ages 6 months and 12 months (Figure 2). Moreover, we dichotomously analyzed the data and observed that any breastfeeding continued during the first 6 months after birth was associated with reduced neurodevelopmental delay at ages 6 months [aOR: 0.79 (95% CI: 0.73–0.84)] and 12 months [0.80 (0.75–0.85)] (Table 2). Any breastfeeding during the first 12 months of life was similarly associated with reduced neurodevelopmental delay at age 12 months [0.80 (0.76–0.84)]. Furthermore, exclusive breastfeeding that continued during the first 3 months, but not the first 6 months, was associated with neurodevelopmental delay at age 12 months (0.84 [0.80–0.88]).

To conduct sibling pair analysis, we extracted data from pairs of siblings who both underwent a neurodevelopmental assessment at 6 months old (3220 pairs) and 12 months old

(3117 pairs). Among these children, we further selected sibling pairs who were discordant for various breastfeeding statuses (Table 3). Few variations existed in the statuses between pairs; therefore, the number of selected pairs was relatively small, varying from 412 pairs (824 children) to 800 pairs (1600 children), based on age (3 months, 6 months, or 12 months) and type (any breastfeeding or exclusive breastfeeding). Among these combinations, the adjusted conditional logistic regression model for 699 sibling pairs (1398 children) revealed that any breastfeeding during the first 12 months was significantly associated with reduced neurodevelopmental delay at this age (0.59 [0.39–0.91]). The mean breastfeeding duration was 12 months in the sibling who was continuously breastfed and 7.8 ± 2.9 months in the sibling who was not. To clarify how differently siblings were breastfed during the first year of life, we classified 3117 pairs whose neurodevelopmental assessment at 12 months old was recorded into 3 groups: "both" (both children were breastfed), "discordant" (only one child was breastfed), and "neither" (neither child was breastfed) (Figure 3). The number of discordant pairs increased from 43 (1.4%) pairs by the first month of life to 389 (12.5%) pairs by 6 months and 666 (21.4%) pairs by 12 months. Moreover, exclusive breastfeeding was not significantly associated with reduced neurodevelopmental delay at any age (Table 3).

DISCUSSION

The present study investigated the relationship between breastfeeding and child neurodevelopment during the first year of life. Ordinary logistic regression analyses demonstrated that any breastfeeding continued during the first 6 months and 12 months of life and exclusive breastfeeding during the first 3 months of life were significantly associated with reduced neurodevelopmental delay. Our sibling pair analysis revealed that any breastfeeding during the first year of life is a significant indicator of neurodevelopmental differences between siblings.

The association that we observed between breastfeeding and brain functions has repeatedly been reported in observational, meta-analysis, and randomized controlled studies.[3,4,7,8,31] In these studies, the results were heterogeneously adjusted for various parental and environmental confounders. However, no matter how many measured confounders are included, unmeasured confounding factors always exist. Hence, we opted for sibling pair analysis, which controls for all factors shared by siblings from the same mother.[17] We observed a significant association between breastfeeding and neurodevelopment at 12 months old. Our findings further support the World Health Organization's recommendations concerning continued breastfeeding beyond 6 months old.[2] The reason for our significant results is unlikely to be explained simply by the sufficient number of our discordant pairs of siblings (1398 children), which is comparable to the number in previous studies[9,15,16] reporting null findings (1046, 1090, and 1773 children). A possible explanation is that we assessed neurodevelopment in the first year of life, whereas the previous studies assessed it at 4–14 years old. A randomized control study showed that the beneficial effects of breastmilk on cognitive development decrease with advancing age; thus, other environmental and genetic factors may become more important as children age.[18]

In contrast to any breastfeeding, exclusive breastfeeding had no or a rather slightly higher association with neurodevelopmental delay in our study. Research on the association between exclusive breastfeeding and cognitive development is relatively scarce and has yielded inconsistent results: some studies[32,33] report positive effects of exclusive breastfeeding on neurodevelopment, whereas other studies[34-36] report limited or rather negative effects. The reason for the reduced effects of exclusive breastfeeding versus that of any breastfeeding is not well understood. Some researchers suggest that breast milk may not meet the full requirements for energy and micronutrients such as iron and zinc, which all have important roles in the developing brain,[37] of the average infant at 6 months old.[38] Withholding formula milk and complementary food until age 6 months may negate the beneficial effects of breastfeeding.

In this study, the number of pairs who were discordantly breastfed in the first year of life increased with age, with the least discordance being at 1 month old, at which point 98.2% of the sibling pairs were both breastfed. This finding suggests that most mothers breastfeed their children in early infancy but discontinue later at different times for each sibling. Thus, the association between breastfeeding and neurodevelopment is probably related more to breastfeeding late into year 1 rather than breastfeeding early. By contrast, a previous randomized controlled trial[31] in which participants were randomly assigned to a breastfeeding promotion intervention group demonstrated that discordance in breastfeeding between an intervention group and control group was larger in early infancy than later in the first year of life. Late discordance such as that in the present study may be common in studies with an observational design. The brain is more sensitive to environmental factors earlier in life; therefore, the discordance later in life may produce less divergent impacts on brain development between siblings. This factor may explain, at least partially, the null results of sibling comparison in previous observational studies.[9,15,16]

Strengths and limitations

To our knowledge, this study is the largest birth cohort study examining the association between breastfeeding and brain function. We conducted sibling pair analyses with a sufficient number of participants from this large cohort, which enabled us to have strong control over sibling-shared parental and environmental factors. Monthly information on feeding methods was precisely obtained *via* successive questionnaires at 1 month, 6 months, and 12 months old, which yielded a much smaller risk of recall bias than that of previous sibling pair studies.[9,15,16]

The current study had some limitations. The information was largely obtained from self-administered questionnaires. In particular, the identified neurodevelopmental delay may be somewhat equivocal because it relied solely on responses on the parent-reported screening test of Japanese version of ASQ-3. Furthermore, even in sibling pair analysis, other confounding factors such as environmental factors may be responsible for the differences because siblings do not share all environmental factors and shared environments may not always be stable.[17]

CONCLUSION

The present study demonstrated for the first time, by using sibling pair analysis, an association of continuous breastfeeding with reduced neurodevelopmental delay at 1 year old. This less-confounded association provides a more persuasive argument for public health practitioners and policymakers to promote breastfeeding continuation, at least during the first year of life. The ongoing JECS cohort may reveal how long the observed beneficial effects will persist in later life.

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Contributors

M. Sanefuji conceived, designed the study, analysed the data, interpreted the results and wrote the manuscript. AS, M. Shimono and MO interpreted the results and critically reviewed the manuscript. Y. Sonoda, MT, YI, RS and Y. Sakai critically reviewed the manuscript. SH analysed the data, interpreted the results and critically reviewed the manuscript. KK and SO directed the study and critically reviewed the manuscript.

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Data sharing

The dataset used in this study is available only to researchers who are approved by the Ministry of the Environment, Japan.

Appendix

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Table 1. Baseline characteristics of the children

	Full sample (n = 77 119)	Missing	Sibling sample (n = 7055)	Missing	Effect size [a]
Boy, no. (%)	39 350 (51.0)	0	3552 (50.3)	0	0.00
Gestational age (wk.), mean (SD)	39.5 (1.1)	0	39.5 (1.1)	0	0.00
Birth weight (g), mean (SD)	3062 (365)	0	3079 (360)	0	0.01
Maternal age (y), mean (SD)	31.3 (4.9)	4	29.8 (4.6)	0	0.09
Maternal smoking status during pregnancy, no. (%)	12 424 (16.3)	858	1062 (15.2)	58	0.01
Maternal education, no. (%)		700		49	0.02
Junior high school	3029 (4.0)		310 (4.4)		
High school	56 180 (73.5)		5264 (75.1)		
University/graduate school	17 210 (22.5)		1432 (20.4)		
Paternal education, no. (%)		1111		62	0.03
Junior high school	4960 (6.5)		541 (7.7)		
High school	44 973 (59.2)		4381 (62.6)		
University/graduate school	26 075 (34.3)		2071 (29.6)		
Family income, no. (%)		5454		427	0.03
Low (<4,000,000 JPY)	28 012 (39.1)		2836 (42.8)		
Middle (4,000,000–5,999,999 JPY)	24 070 (33.6)		2189 (33.0)		
High (≥6,000,000 JPY)	19 583 (27.3)		1603 (24.2)		
Complementary food before 6 months, no. (%)	34 126 (44.9)	1175	3194 (45.9)	95	0.01
Home speech stimulation at 1 month, no. (%)	62 400 (81.1)	214	5611 (79.7)	17	0.01
Home speech stimulation at 12 months, no. (%)	39 175 (51.0)	273	3398 (48.3)	21	0.02
Any breastfeeding (1 month), no. (%)	76 167 (98.8)	0	6976 (98.9)	0	0.00
Any breastfeeding (6 months), no. (%)	63 296 (82.1)	0	5713 (81.0)	0	0.01
Any breastfeeding (12 months), no. (%)	49 672 (64.4)	0	4148 (58.8)	0	0.04
Exclusive breastfeeding (3 months), no. (%)	30 049 (39.6)	1175	3031 (43.5)	95	0.03
Exclusive breastfeeding (6 months), no. (%)	15 447 (20.3)	1175	1507 (21.7)	95	0.01
Neurodevelopmental delay at 6 months, no. (%)	6162 (8.4)	3769	559 (8.3)	322	0.00
Neurodevelopmental delay at 12 months, no. (%)	10 442 (14.6)	5381	888 (13.4)	443	0.01

[a] The difference between sibling samples versus the rest (n = 70 064). Effect sizes are calculated as *phi*/Cramer's *V* and *r*, using chi-square and Student's t tests for the categorical and numerical variables, respectively. SD, standard deviation; JPY, Japanese yen

Table 2. Neurodevelopmental delay in association with any or exclusive breastfeeding for the full sample (n = 77 119)

	Neurodevelopmental delay at 6 months			Neurodevelopmental delay at 12 months		
	Number	cOR (95% CI)	aOR (95% CI) [a,b]	Number	cOR (95% CI)	aOR (95% CI) [a,b,c]
Any breastfeeding						
During the first 6 months						
No	1263/12 967 (9.7%)	1 (reference)	1 (reference)	2091/12 735 (16.4%)	1 (reference)	1 (reference)
Yes	4899/60 383 (8.1%)	**0.82 (0.77–0.87)**	**0.79 (0.73–0.84)**	8351/59 003 (14.2%)	**0.84 (0.80–0.88)**	**0.80 (0.75–0.85)**
During the first 12 months						
No	—	—	—	4061/25 303 (16.0%)	1 (reference)	1 (reference)
Yes	—	—	—	6381/46 435 (13.7%)	**0.83 (0.80–0.87)**	**0.80 (0.76–0.84)**

	Neurodevelopmental delay at 6 months			Neurodevelopmental delay at 12 months		
	Number	cOR (95% CI)	aOR (95% CI)[a]	Number	cOR (95% CI)	aOR (95% CI) [a,c]
Exclusive breastfeeding						
During the first 3 months						
No	3794/43 558 (8.7%)	1 (reference)	1 (reference)	6637/42 648 (15.6%)	1 (reference)	1 (reference)
Yes	2273/28 686 (7.9%)	**0.90 (0.85–0.95)**	0.94 (0.89–1.00)	3664/28 051 (13.1%)	**0.82 (0.78–0.85)**	**0.84 (0.80–0.88)**
During the first 6 months						
No	4768/57 508 (8.3%)	1 (reference)	1 (reference)	8228/56 374 (14.6%)	1 (reference)	1 (reference)
Yes	1299/14 736 (8.8%)	**1.07 (1.00–1.14)**	1.05 (0.98–1.12)	2073/14 325 (14.5%)	0.99 (0.94–1.04)	0.96 (0.91–1.01)

[a] Adjusted for sex, gestational age, birthweight, mother's age, maternal smoking, maternal and paternal education, family income and home speech stimulation at 1 month. [b] Adjusted further for the introduction of complementary food. [c] Adjusted further for home speech stimulation at 12 months. Boldface represents statistical significance (*P* < 0.05). Abbreviations: cOR, crude odds ratio; aOR, adjusted odds ratio; CI, confidence interval

Table 3. Neurodevelopmental delay in association with any or exclusive breastfeeding in discordant pairs from sibling sample (n = 7055)

	Neurodevelopmental delay at 6 months			Neurodevelopmental delay at 12 months		
	Number	cOR (95% CI)	aOR (95% CI)[a,b]	Number	cOR (95% CI)	aOR (95% CI)[a,b,c]
Any breastfeeding						
During the first 6 months						
No	36/412 (8.7%)	1 (reference)	1 (reference)	65/414 (15.7%)	1 (reference)	1 (reference)
Yes	29/412 (7.0%)	0.78 (0.46–1.32)	0.63 (0.32–1.23)	55/414 (13.3%)	0.78 (0.50–1.21)	0.85 (0.51–1.43)
During the first 12 months						
No		-	-	100/699 (14.3%)	1 (reference)	1 (reference)
Yes		-	-	78/699 (11.2%)	0.71 (0.51–1.01)	**0.59 (0.39–0.91)**

	Neurodevelopmental delay at 6 months			Neurodevelopmental delay at 12 months		
	Number	cOR (95% CI)	aOR (95% CI)[a]	Number	cOR (95% CI)	aOR (95% CI)[a,c]
Exclusive breastfeeding						
During the first 3 months						
No	60/800 (7.5%)	1 (reference)	1 (reference)	96/755 (12.7%)	1 (reference)	1 (reference)
Yes	62/800 (7.8%)	1.04 (0.71–1.53)	0.94 (0.61–1.44)	97/755 (12.8%)	1.02 (0.72–1.44)	0.98 (0.66–1.46)
During the first 6 months						
No	51/657 (7.8%)	1 (reference)	1 (reference)	70/633 (11.1%)	1 (reference)	1 (reference)
Yes	49/657 (7.5%)	0.95 (0.61–1.47)	0.75 (0.44–1.29)	83/633 (13.1%)	1.27 (0.87–1.85)	1.17 (0.74–1.83)

[a] Adjusted for sex, gestational age, birthweight, sibling order, maternal smoking, and home speech stimulation at 1 month. [b] Adjusted further for the introduction of complementary food. [c] Adjusted further for home speech stimulation at 12 months. Boldface represents statistical significance (*P* < 0.05). Abbreviations: cOR, crude odds ratio; aOR, adjusted odds ratio; CI, confidence interval

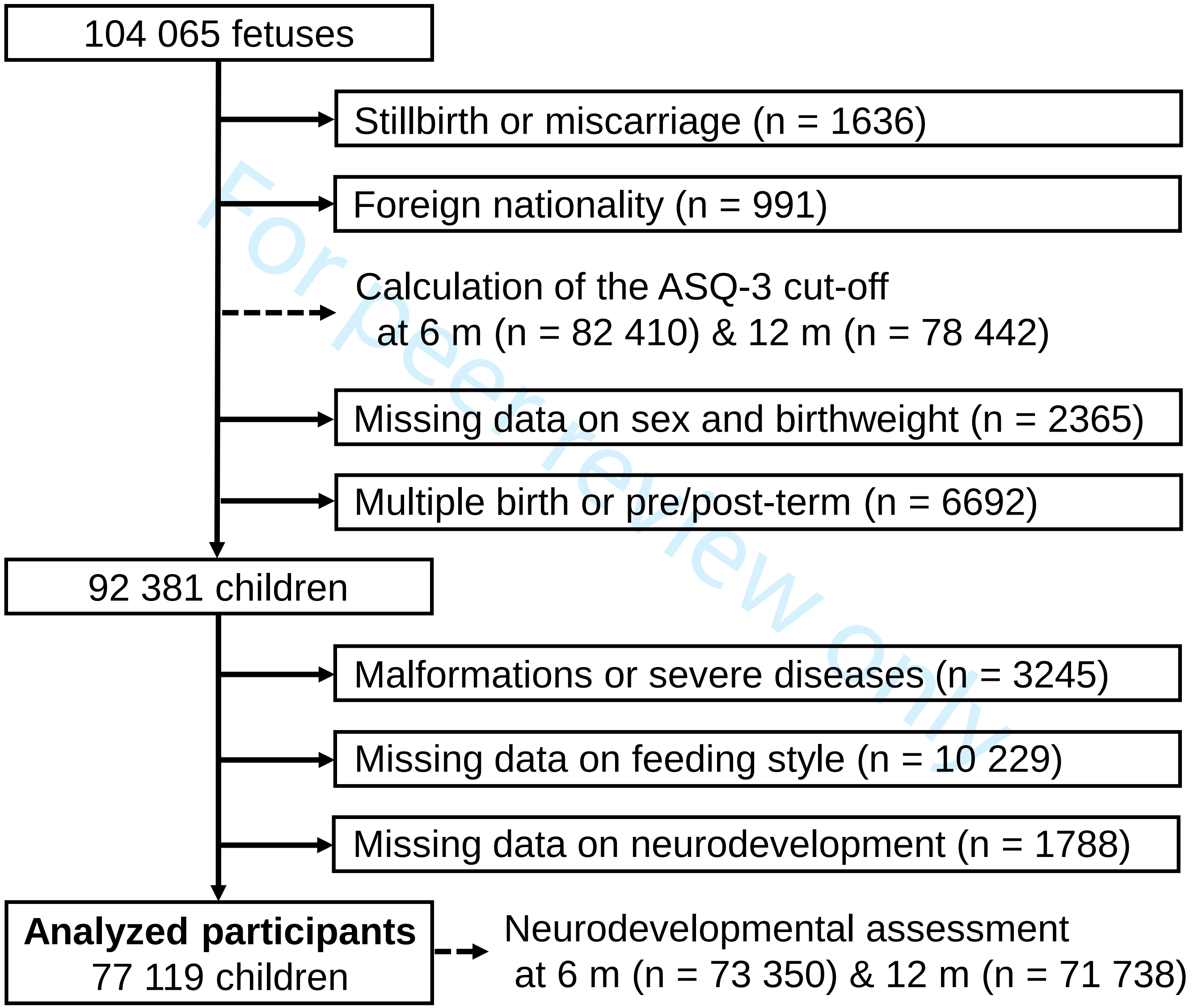
Figure Legends

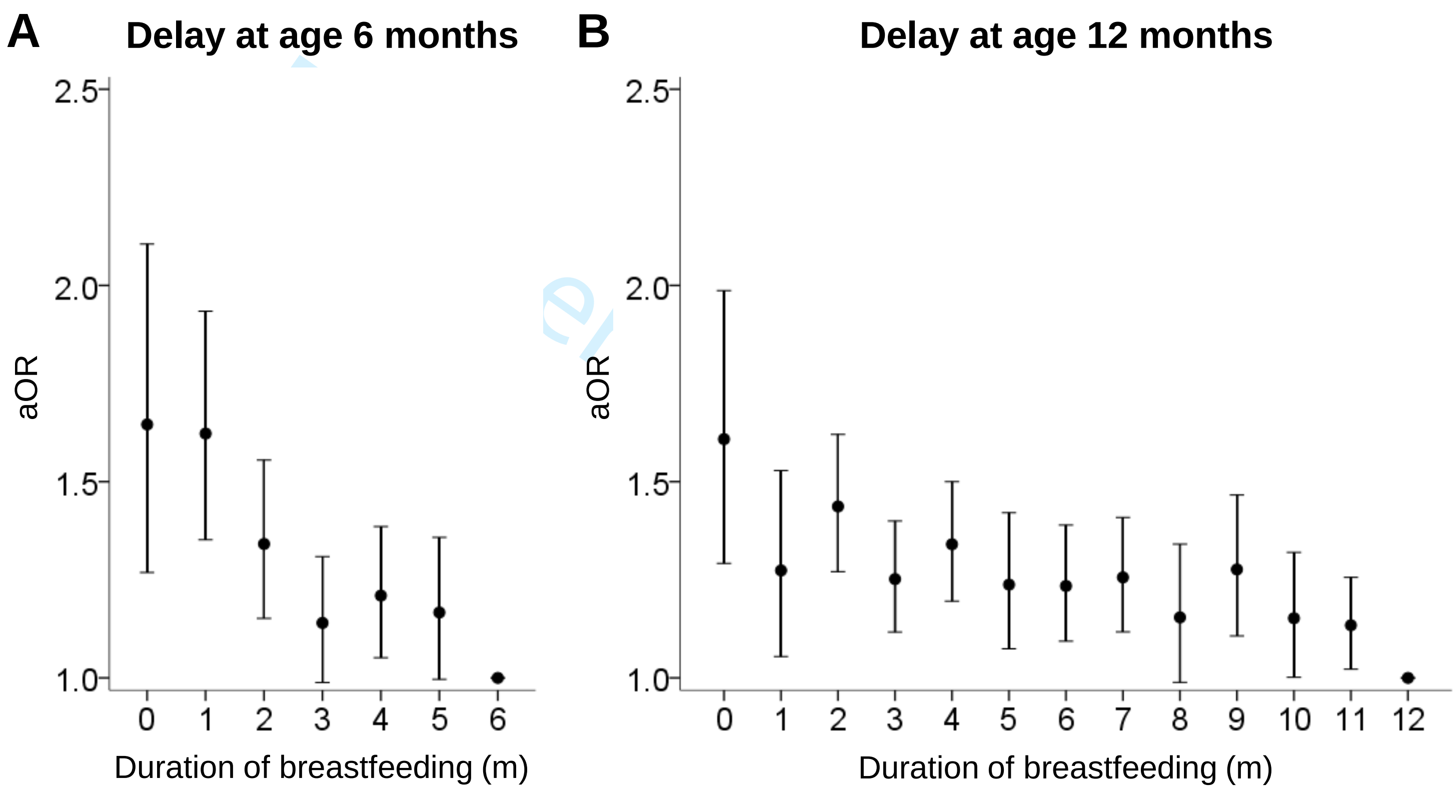
Figure 1. Flowchart of participant selection.

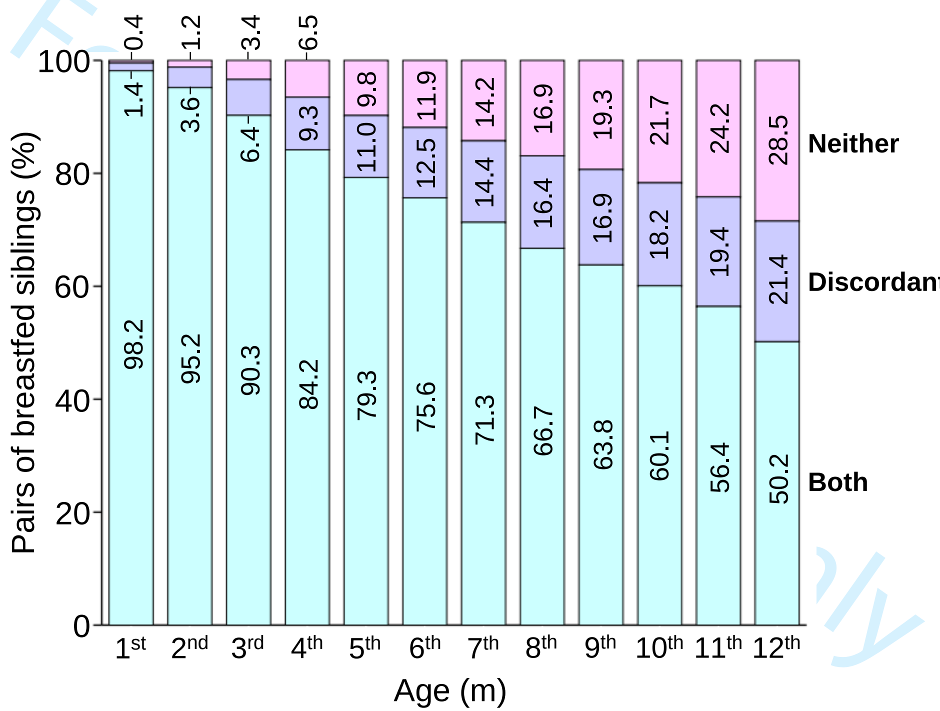
ASQ-3, Ages and Stages Questionnaires, third edition

Figure 2. Neurodevelopmental delay relative to the duration of any breastfeeding. The circles indicate aORs; whiskers, 95% CIs. Each aOR is referenced to the breastfeeding duration: (A) 6 months or (B) 12 months. The adjusted covariates correspond to "any breastfeeding" in Table 2.

Figure 3. Pairs of siblings who were both breastfed, discordantly breastfed, or neither breastfed with respect to each month of life (n = 3117).


104 065 fetuses
Stillbirth or miscarriage (n = 1636)
Foreign nationality (n = 991)
Calculation of the ASQ-3 cut-off at 6 m (n = 82 410) & 12 m (n = 78 442)
Missing data on sex and birthweight (n = 2365)
Multiple birth or pre/post-term (n = 6692)
92 381 children
Malformations or severe diseases (n = 3245)
Missing data on feeding style (n = 10 229)
Missing data on neurodevelopment (n = 1788)
Analyzed participants
77 119 children
Neurodevelopmental assessment at 6 m (n = 73 350) & 12 m (n = 71 738)


A
Delay at age 6 months
B
Delay at age 12 months
aOR
2.5
2.0
1.5
1.0
0 1 2 3 4 5 6
0 1 2 3 4 5 6 7 8 9 10 11 12
Duration of breastfeeding (m)
Duration of breastfeeding (m)


Pairs of breastfed siblings (%)
0
20
40
60
80
100
0.4
1.2
3.4
6.5
1.4
3.6
6.4
9.3
98.2
95.2
90.3
84.2
9.8
11.0
79.3
11.9
12.5
75.6
14.2
14.4
71.3
16.9
16.4
66.7
19.3
16.9
63.8
21.7
18.2
60.1
24.2
19.4
56.4
28.5
21.4
50.2
Neither
Discordant
Both
1st 2nd 3rd 4th 5th 6th 7th 8th 9th 10th 11th 12th
Age (m)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract Confirmed (page 3 of 30)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Confirmed (page 3)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Confirmed (pages 6 & 7)
Objectives	3	State specific objectives, including any prespecified hypotheses Confirmed (page 7)
Methods		
Study design	4	Present key elements of study design early in the paper Confirmed (page 7)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Confirmed (pages 7 & 8)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Confirmed (page 8)
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed Confirmed (page 11)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Confirmed (pages 9-11)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Confirmed (pages 8)
Bias	9	Describe any efforts to address potential sources of bias Confirmed (page 12, Table 1)
Study size	10	Explain how the study size was arrived at Confirmed (page 8)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Confirmed (pages 9 & 10)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding Confirmed (pages 11 & 12)
		(*b*) Describe any methods used to examine subgroups and interactions Confirmed (page 11)
		(*c*) Explain how missing data were addressed Confirmed (page 8, Figure 1, Table 1)
		(*d*) If applicable, explain how loss to follow-up was addressed Confirmed (page 8, Figure 1)
		(*e*) Describe any sensitivity analyses Not applicable

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Confirmed (pages 8 & 12, Figures 1-3, Table 1)
		(b) Give reasons for non-participation at each stage Confirmed (pages 13 & 14)
		(c) Consider use of a flow diagram Confirmed (Figure 1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Confirmed (Table 1)
		(b) Indicate number of participants with missing data for each variable of interest Confirmed (Table 1)
		(c) Summarise follow-up time (eg, average and total amount) Confirmed (page 8)
Outcome data	15*	Report numbers of outcome events or summary measures over time Confirmed (page 12, Table 1)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Confirmed (pages 13 & 14, Tables 2 & 3)
		(*b*) Report category boundaries when continuous variables were categorized Confirmed (pages 13)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Confirmed (pages 13 & 14)
Discussion		
Key results	18	Summarise key results with reference to study objectives Confirmed (page 14)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Confirmed (pages 17)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Confirmed (pages 15 & 16)
Generalisability	21	Discuss the generalisability (external validity) of the study results Confirmed (page 15)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based Confirmed (page 18)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Breastfeeding and infant development in a cohort with sibling pair analysis: the Japan Environment and Children's Study

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Breastfeeding and infant development in a cohort with sibling pair analysis: the Japan Environment and Children's Study

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ABSTRACT

Objectives

To investigate the association between breastfeeding and infant development during the first year of life using sibling comparison.

Design

Nationwide prospective birth cohort study with sibling pair analysis.

Setting

15 regional centres that participated in the Japan Environment and Children's Study.

Participants

This study included 77 119 children (singleton, term birth and no malformation/severe diseases) whose mothers were registered between January 2011 and March 2014, including 3 521 duos or trios of siblings.

Primary outcome measures

The primary outcome was developmental delay at 6 and 12 months of age, assessed using the Japanese translation of the Ages and Stages Questionnaires, third edition. Multivariable regression analyses adjusted for confounders were performed to estimate the risk ratios of delay associated with any or exclusive breastfeeding. Pairs of siblings discordant for statuses were selected, and conditional regression analyses were conducted with a matched cohort design.

Results

Developmental delay was identified in 6162 (8.4%) and 10 442 (14.6%) children at 6 and 12 months of age, respectively. Any breastfeeding continued until 6 months or 12 months old was associated with reduced developmental delay at 12 months of age (adjusted risk ratio [95% confidence interval]: 0.81 [0.77 to 0.85] and 0.81 [0.78 to 0.84], respectively). Furthermore, exclusive breastfeeding until 3 months was associated with reduced developmental delay at 12 months of age (0.86 [0.83 to 0.90]). In sibling pair analysis, the association between any breastfeeding until 12 months and reduced developmental delay at 12 months of age persisted (0.64 [0.43 to 0.93]).

Conclusions

The present study demonstrated the association of continuous breastfeeding with reduced developmental delay at 1 year of age using sibling pair analysis, in which unmeasured confounding factors are still present but less included. This may provide an argument to promote breastfeeding continuation.

Strengths and limitations of this study

- This study is the largest birth cohort study that investigated the association between breastfeeding and infant development.
- The association was examined using not only ordinary multivariable regression analysis but also sibling comparison, which strongly controls for sibling-shared factors.
- Monthly feeding status was collected at child's age of 1 month, 6 and 12 months, minimizing the risk of recall bias.
- Developmental delay was determined by a parent-reported screening test and thus may be equivocal.
- The results could not eliminate the possibility that the association still could be explained by reverse causation because the reason for cessation of breastfeeding was not known.

INTRODUCTION

Since 1929, the beneficial effects of breastfeeding on brain development have been repeatedly demonstrated.[1-4] Many observational studies[5-8] demonstrate that breastfeeding is associated with better cognitive outcomes, including neurodevelopment, language, and intelligence. In these studies, however, the causation remains unclear because the reason for cessation of breastfeeding is not known. Furthermore, this association can be produced by differences in demographic, socioeconomic, and environmental factors between mothers who breastfeed and those who do not.[9-12] In high-income countries, mothers with higher levels of education, social position, income, and intelligence are more inclined to breastfeed and to do so more exclusively and for a longer duration. Thus, their children are more likely to have higher cognitive functions, which can result in a superficial association between breastfeeding and better child cognition. In previous studies, the association disappeared or became highly diminished after controlling for confounders, especially maternal intelligence.[9, 13, 14] Nonetheless, a recent meta-analysis concluded that breastfeeding was significantly associated with higher cognitive abilities, even after adjusting for such confounding factors.[3]

After explicitly controlling for these measured factors, unmeasured—even unknown—confounders such as parental characteristics and child-rearing practices remained. To further control for these confounders, previous studies[9, 15-17] conducted sibling pair analysis in investigating the association of breastfeeding with child cognitive outcomes. These analyses focused on siblings pairs who were discordant for breastfeeding exposure. A sibling pair from the same mother largely shares parental and environmental factors. Thus, the effects of these confounders can be cancelled out when the pair is matched in the analysis. However, on this topic, sibling pair analysis is challenging because little variation in breastfeeding often exists between siblings, which may reduce statistical power and erroneously cause null findings.[17] To our knowledge, only three studies[9, 15, 16] have examined the association between breastfeeding and cognitive functions using this method, and these studies all produced statistically null effects. The reason for the null results remains unclear. However, these findings may be accounted for by the study designs: data on feeding status were collected only once within 1 year[9] or 2 years[15] after a child's birth or in adolescence.[16]

The goal of the current study was to investigate the association between breastfeeding and child development during the first year of life by using data from the Japan Environment and Children's Study (JECS). This nationwide birth cohort study includes >100,000 children and thus enables sibling pair analysis with a sufficient number of participants. The monthly status of breastfeeding was collected repeatedly in the first year of life, thereby minimizing the risk of recall bias. The beneficial effects of breastfeeding on cognitive development decrease as children age;[18] therefore, investigating the association between breastfeeding and cognitive development during early childhood has the advantage of allowing researchers to infer the role of breastfeeding on the developing brain.

METHODS

Design

The JECS is a nationwide, multicenter, prospective birth cohort study funded by the Ministry of Environment, Japan. The details of the study design have been described elsewhere.[19, 20] Briefly, pregnant participants were registered between January 2011 and March 2014 in 15 regional centers covering a wide geographical area in Japan. During pregnancy, data on demographics, smoking, alcohol, education, and socioeconomic statuses were obtained during the first and second/third trimesters by using self-administered questionnaires. Detailed information regarding the mother and child was obtained from medical records transcripts during the first trimester, at the time of delivery, and when the child was 1 month old. After delivery, data on feeding style, use of complementary foods, developmental status, and affected diseases were collected at ages 1 and 6 months and every 6 months until the child was 6 years old, and then twice a year thereafter *via* self-reported questionnaires completed by the parents.

The JECS protocol was reviewed and approved by the Ministry of Environment's Institutional Review Board for Epidemiological Studies (No.100910001) and by the ethics committees of all participating institutions (No.2019-070). The ethical approval for this study was an extension of the ethical approval for the JECS protocol. Written informed consent was obtained from all parents.

Participants

In this study, we used the fixed dataset "jecs-an-20180131" that was released in March 2018. This dataset contains all available data extracted from the aforementioned questionnaires and records until a child was 12 months old. The data for 104 065 fetuses from 103 062 pregnancies were linked to the respective maternal data. The participants selected were 92 381 live-born singleton children, delivered at term (≥37 gestational weeks and <42 gestational weeks), of parents of Japanese nationality and for whom information on sex and birthweight had been recorded (Figure 1). Of these children, those who had malformations or severe diseases, or who had missing information on feeding style during the first year of life or development at 6 months and 12 months old were excluded. After these exclusions, the data of 77 119 children were included in our analysis.

Exposure

The main exposure factor was breastfeeding. Mothers were asked to fill in the monthly feeding status of their child by using questionnaires when the child was 1 month, 6 months, and 12 months old. This information included whether the child was breastfed, formula-fed, or both. The questionnaire administered when the child was 12 months old also queried when complementary food was first started. Breastfeeding duration indicated how long a child was breastfed from birth, irrespective of concurrent consumption of formula milk. We also dichotomously assessed whether or not a child continued (1) any breastfeeding until 6 months old, (2) any breastfeeding until 12 months, (3) exclusive breastfeeding until 3 months, and (4) exclusive breastfeeding until 6 months. Breastfeeding was “exclusive” if the child consumed only breastmilk—and nothing else (no consumption of formula milk or complementary foods) during these periods.

For sibling pair analysis, we selected pairs who were discordant on the status of any breastfeeding or exclusive breastfeeding. When discordance was observed among three siblings (e.g., only one of the three children was breastfed), we randomly selected one of the two siblings who were not breastfed and then paired the selected one with the breastfed sibling.

Outcome

The outcome was developmental delay measured at 6 months and 12 months old, using the Japanese translated version of the Ages and Stages Questionnaires: A Parent-Completed Monitoring System (ASQ), third edition. This version was prepared through a back-translation procedure and was approved by the publisher of the original English version.[21] The ASQ can identify infants or young children who need further developmental assessment to determine whether they are eligible for early intervention. The findings of the questionnaire basically agree with those of professionally administered developmental batteries.[22, 23] It has been used in clinical and research settings and translated into several languages.[24-27] The ASQ assesses five developmental domains. For each domain, six skills are described to which parents answer "yes," "sometimes," or "not yet," depending on whether their child is demonstrating the described skill. The responses are converted to points, with "yes" receiving 10 points; "sometimes", 5 points; and "not yet", 0 points. The child's score for each developmental domain is the sum of all points received for the items under that domain and ranges from 0 to 60 points. The cut-off score for each domain was defined as two standard deviations below the mean score of large standardized samples in the United States of America. A child was defined as having a developmental delay if a score was at or below the cut-off level in any developmental domain. When the cut-off scores of the original English version were used in our population, an excessive number of children were classified as having a developmental delay (47.4% and 34.6% for 6 months and 12 months, respectively). Although preliminary cut-off scores of the Japanese translation were recently proposed,[28] these were not recommended to be used with confidence before 24 months old because of very limited sample sizes. Therefore, the cut-off scores were determined by using the same methodologies used in the original version, based on available data at ages 6 months (n = 82 410) and 12 months (n = 78 442) (Figure 1), which would represent the general Japanese population. As a continuous variable, in addition, total score of ASQ was defined as the sum of the scores for the five domains, ranging from 0 to 300 points.

Statistical analysis

To assess the association of breastfeeding with child development, we conducted multivariable quasi-Poisson regression analyses for dichotomous dependent variables, and multiple linear regression analyses for continuous dependent variables. The adjusted covariates were i) sex, ii) gestational age, iii) birthweight, iv) mother's age, v) maternal smoking status during pregnancy, as recorded in the first trimester, vi) maternal alcohol consumption during pregnancy, as recorded in the second trimester, vii) maternal and viii) paternal education level (junior high school, high school, and university or graduate school), ix) annual family income (<4 000 000; 4 000 000–5 999 999; ≥6 000 000 JPY), x) introduction of complementary foods before 6 months old, and xi) home speech stimulation at 1 month (whether a mother did or did not talk to her baby habitually: yes/no). The "home speech stimulation" covariate was used instead of the Home Observation for Measurement of the Environment scale,[29] which is not employed in the JECS.

For sibling pair analysis, we conducted conditional logistic regression analyses with 1:1 matched cohort data of sibling pairs whose dichotomous statuses of breastfeeding were discordant.[30] We reported adjusted relative risks (aRRs) with 95% confidence intervals (CIs) that were converted from odds ratios using an established method.[31, 32] We also used a longitudinal linear mixed model, in which fixed effects were age of ASQ assessment (6 *vs.* 12 months old), duration of breastfeeding, and the interaction term between them, with random intercept for sibling. The adjusted covariates were as follows: i) sex, ii) gestational age, iii) birthweight, iv) order of siblings in the discordant pair, v) maternal smoking status, vi) maternal alcohol consumption, vii) complementary food introduction, and viii) home speech stimulation at 1 month old. All statistical analyses were conducted using R software (version 3.5.0). In the R package, we used "survival" (version 3.2.7) for conditional logistic regression model and "lme4" for longitudinal linear mixed model. The level of significance was $P = 0.05$.

Patient and public involvement

No participants were involved in creating the research question or the outcome measures, nor were they involved in developing plans for recruitment, design or implementation of the study. No participants were asked to provide advice on the interpretation or writing up of the results. There are plans to disseminate the results of the research to study participants and the general public. Participants were thanked in the acknowledgments.

RESULTS

The baseline characteristics of 77 119 children are summarized in Table 1. Nearly all (76 167, 98.8%) children were started on any breastmilk during their first month of life. Any breastfeeding was continued until ages 6 and 12 months in 82.1% and 64.4% of children, respectively. Exclusive breastfeeding was continued until ages 3 and 6 months in 39.6% and 20.3% of children, respectively. Developmental delay was identified in 8.4% and 14.6% of children at 6 months and 12 months old, respectively. The sibling cohort included 3521 sibling sets (7055 children) in total: 3508 duos (7016 children) and 13 trios (39 children). The characteristics of the sibling sample were substantially similar to those of the full sample. Nevertheless, the sibling sample appeared to have weak tendencies towards younger maternal age, lower paternal education, lower family income, lower rates for any breastfeeding until 12 months old, and higher rates for exclusive breastfeeding until 3 months.

For the full sample (n = 77 119), we conducted multivariable regression analyses, while adjusting for confounders, to examine developmental delay in relation to various types of breastfeeding exposures. When breastfeeding was treated as dichotomous variables, quasi-Poisson models reveled that any breastfeeding continued until 6 months was associated with reduced developmental delay at ages 6 months [aRR: 0.81 (95% CI: 0.76 to 0.86)] and 12 months [0.81 (0.77 to 0.85)] (Table 2). Any breastfeeding until 12 months was similarly associated with reduced developmental delay at age 12 months [0.81 (0.78 to 0.84)]. Any breastfeeding was similarly continued until 12 months old between children with (77.4%) and without developmental delay (78.6%) at 6 months old (Figure S1), arguing against the possibility that developmental delay *per se* interrupted the continuation of breastfeeding. When developmental delay was not observed at 6 months old, it is more likely to occur newly at 12 months in children who discontinued breastfeeding by 12 months old than those continued it while delay at 6 months resolved more often in children who continued breastfeeding (Figure S2). Furthermore, exclusive breastfeeding that continued until 3 months old, but not until 6 months, was associated with developmental delay at age 12 months (0.86 [0.83 to 0.90], Table 2). When breastfeeding was treated as continuous variables, multiple linear regression model demonstrated that duration of any or exclusive breastfeeding was positively associated with increased total ASQ scores at 6 and 12 months old (Table 3).

To conduct sibling pair analysis, we extracted data from pairs of siblings who both underwent a developmental assessment at 6 months old (3220 pairs) and 12 months old (3117 pairs). Among these children, we further selected sibling pairs who were discordant for various breastfeeding statuses (Figure 1 and Table 4). Few variations existed in the statuses between pairs; therefore, the number of selected pairs was relatively small, varying from 412 pairs (824 children) to 800 pairs (1600 children), based on age (3 months, 6 months, or 12 months) and type (any breastfeeding or exclusive breastfeeding). Among these combinations, the adjusted conditional regression model for 699 sibling pairs (1398 children) revealed that any breastfeeding until 12 months was significantly associated with reduced developmental delay at this age (0.64 [0.43 to 0.93]). The mean breastfeeding duration was 12 months in the sibling who was continuously breastfed and 7.8 ± 2.9 months in the sibling who was not. Moreover, exclusive breastfeeding was not significantly associated with reduced developmental delay at any age. In sibling pairs discordant for any breastfeeding until 12 months, when the first-born children continued breastfeeding, the second-born, who discontinued it, had a tendency for developmental delay at 12 months; when the first born discontinued breastfeeding, the second showed a reduced tendency (Figure S3). In sibling pairs who were discordant for maternal smoking, a proxy for socioeconomical status at that time, any breastfeeding was similarly continued until 12 months old between children whose mothers had smoking (52.9%) *vs.* no smoking (54.5%) during pregnancy (Figure S4). When breastfeeding was treated as continuous variables, longitudinal linear mixed model revealed that duration of any, but not exclusive, breastfeeding was associated with increased total ASQ score (Table 5).

To clarify how differently siblings were breastfed during the first year of life, we classified 3117 pairs whose developmental assessment at 12 months old was recorded into 3 groups: "both" (both children were breastfed), "discordant" (only one child was breastfed), and "neither" (neither child was breastfed) (Figure 2). The number of discordant pairs increased from 43 (1.4%) pairs at the first month of life to 389 (12.5%) pairs at 6 months and 666 (21.4%) pairs at 12 months.

DISCUSSION

The present study investigated the relationship between breastfeeding and child development during the first year of life. Ordinary logistic regression analyses demonstrated that any breastfeeding continued until 6 or 12 months old, and exclusive breastfeeding until 3 months were significantly associated with reduced developmental delay. In the sibling pair analysis, only the association between any breastfeeding until 12 months old and reduced developmental delay at 12 months old remained significant. The null association of any breastfeeding until 6 months might be explained by failure to detect less developmental variations at 6 months compared with those at 12 months, or involvement of other environmental factors that child had experienced after 6 months old.

The association that we observed between breastfeeding and brain functions has repeatedly been reported in observational, meta-analysis, and randomized controlled studies.[3, 4, 7, 8, 33] In these studies, the results were heterogeneously adjusted for various parental and environmental confounders. However, no matter how many measured confounders are included, unmeasured confounding factors always exist. Hence, we opted for sibling pair analysis, which controls for all factors shared by siblings from the same mother.[17] We observed a significant association between breastfeeding and development at 12 months old. Our findings further support the World Health Organization's recommendations concerning continued breastfeeding beyond 6 months old.[2] The reason for our significant results is unlikely to be explained simply by the sufficient number of our discordant pairs of siblings (1398 children), which is comparable to the number in previous studies[9, 15, 16] reporting null findings (1046, 1090, and 1773 children). A possible explanation is that we assessed child's development in the first year of life, whereas the previous studies assessed it at 4–14 years old. A randomized control study showed that the beneficial effects of breastmilk on cognitive development decrease with advancing age; thus, other environmental and genetic factors may become more important as children age.[18]

The mechanisms underlying the association between breastfeeding and brain development are unclear but may be attributable to its nutrients such as long-chain polyunsaturated fatty acids, hormones and cytokines.[34, 35] Another probable mechanism is mother-infant interaction produced by breastfeeding behaviors.[36] A series of Family Nurture Intervention study have repeatedly demonstrated the importance of early nurturing activities that engage the mother and infant reciprocally in physical, sensory, and emotional experiences in infant development.[37-43] Such nurturing activities *via* breastfeeding may enhance the connection between social motivation and mother-infant relational health,[44] leading to better development.

In contrast to any breastfeeding, exclusive breastfeeding had no significant association with developmental delay in our study. Research on the association between exclusive breastfeeding and cognitive development is relatively scarce and has yielded inconsistent results: some studies report positive effects of exclusive breastfeeding on neurodevelopment,[45, 46] whereas other studies report limited or rather negative effects.[47-49] The reason for the reduced effects of exclusive breastfeeding versus that of any breastfeeding is not well understood. Some researchers suggest that exclusive breastmilk may not meet the full requirements for energy and micronutrients such as iron and zinc, which all have important roles in the developing brain,[50] of the average infant at 6 months old.[51] Withholding formula milk and complementary food until age 6 months may negate the beneficial effects of breastfeeding. Alternatively, such withholding might reflect some unmeasured confounders that adversely related to infant development.

In this study, the number of pairs who were discordantly breastfed in the first year of life increased with age, with the least discordance being at 1 month old, at which point 98.2% of the sibling pairs were both breastfed. This finding suggests that most mothers breastfeed their children in early infancy but discontinue later at different times for each sibling. Thus, the association between breastfeeding and development is probably related more to breastfeeding late into year 1 rather than breastfeeding early. By contrast, a previous randomized controlled trial[33] in which participants were randomly assigned to a breastfeeding promotion intervention group demonstrated that discordance in breastfeeding between an intervention group and control group was larger in early infancy than later in the first year of life. Late discordance such as that in the present study may be common in studies with an observational design. The brain is more sensitive to environmental factors earlier in life; therefore, the discordance later in life may produce less divergent impacts on brain development between siblings. This factor may explain, at least partially, the null results of sibling comparison in previous observational studies.[9, 15, 16]

Strengths and limitations

To our knowledge, this study is the largest birth cohort study examining the association between breastfeeding and brain function. We conducted sibling pair analyses with a sufficient number of participants from this large cohort, which enabled us to have strong control over sibling-shared parental and environmental factors. Monthly information on feeding methods was precisely obtained *via* successive questionnaires at 1 month, 6 months, and 12 months old, which yielded a much smaller risk of recall bias than that of previous sibling pair studies.[9, 15, 16]

The current study also included several limitations. The information was largely obtained from self-administered questionnaires. In particular, the identified developmental delay may be somewhat equivocal because it relied solely on responses on the parent-reported screening test of Japanese version of ASQ. Furthermore, even in sibling pair analysis, other confounding factors such as environmental factors may be responsible for the differences because siblings do not share all environmental factors and shared environments may not always be stable.[17] Finally, there were no data on what factors have contributed to cessation of breastfeeding. Even within a pair of sibling, there could be difference in socioeconomical status, which might alter parent's rearing behaviors and then affect the child's development. If an infant at potential risk of developmental disorders has less preference to breastfeeding, a superficial association can be produced between breastfeeding and better development. Indeed, a meta-analysis demonstrated altered feeding habits in children with attention-deficit/hyperactivity disorder.[52] Although our supplementary analyses rather argued against such possibility, the association between breastfeeding and a reduced risk of developmental delay in our study still could be explained by such reverse causation.

CONCLUSION

The present study demonstrated for the first time, by using sibling pair analysis, an association of continuous breastfeeding with reduced developmental delay at 1 year old. Although the causation should be carefully interpreted in this observational study, the less-confounded association may provide a more persuasive argument for public health practitioners and policymakers to promote breastfeeding continuation, at least during the first year of life. The ongoing JECS cohort may reveal how long the observed beneficial effects will persist in later life.

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Contributors

M. Sanefuji conceived, designed the study, analysed the data, interpreted the results and wrote the manuscript. AS, M. Shimono and MO interpreted the results and critically reviewed the manuscript. Y. Sonoda, MT, YI, RS and Y. Sakai critically reviewed the manuscript. SH analysed the data, interpreted the results and critically reviewed the manuscript. KK and SO directed the study and critically reviewed the manuscript.

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Data sharing

No data are available. For details, see http://www.env.go.jp/chemi/ceh/en/

Ethics Approval

The JECS protocol was reviewed and approved by the Ministry of Environment's Institutional Review Board for Epidemiological Studies (No.100910001) and by the ethics committees of all participating institutions (No.2019-070). The ethical approval for this study was an extension of the ethical approval for the JECS protocol. Written informed consent was obtained from all parents.

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Table 1. Baseline characteristics of the children

	Full sample (n = 77 119)	Missing	Sibling sample (n = 7055)	Missing	Effect size [a]
Boy, no. (%)	39 350 (51.0)	0	3552 (50.3)	0	0.00
Gestational age (wk.), mean (SD)	39.5 (1.1)	0	39.5 (1.1)	0	0.00
Birth weight (g), mean (SD)	3062 (365)	0	3079 (360)	0	0.01
Maternal age (y), mean (SD)	31.3 (4.9)	4	29.8 (4.6)	0	0.09
Maternal smoking during pregnancy, no. (%)	12 424 (16.3)	858	1062 (15.2)	58	0.01
Maternal alcohol during pregnancy, no. (%)	2080 (2.7)	875	231 (3.3)	71	0.01
Maternal education, no. (%)		700		49	0.02
Junior high school	3029 (4.0)		310 (4.4)		
High school	56 180 (73.5)		5264 (75.1)		
University/graduate school	17 210 (22.5)		1432 (20.4)		
Paternal education, no. (%)		1111		62	0.03
Junior high school	4960 (6.5)		541 (7.7)		
High school	44 973 (59.2)		4381 (62.6)		
University/graduate school	26 075 (34.3)		2071 (29.6)		
Family income, no. (%)		5454		427	0.03
Low (<4,000,000 JPY)	28 012 (39.1)		2836 (42.8)		
Middle (4,000,000–5,999,999 JPY)	24 070 (33.6)		2189 (33.0)		
High (≥6,000,000 JPY)	19 583 (27.3)		1603 (24.2)		
Complementary food before 6 months, no. (%)	34 126 (44.9)	1175	3194 (45.9)	95	0.01
Home speech stimulation at 1 month, no. (%)	62 400 (81.1)	214	5611 (79.7)	17	0.01
Any breastfeeding until 1 month, no. (%)	76 167 (98.8)	0	6976 (98.9)	0	0.00
Any breastfeeding until 6 months, no. (%)	63 296 (82.1)	0	5713 (81.0)	0	0.01
Any breastfeeding until 12 months, no. (%)	49 672 (64.4)	0	4148 (58.8)	0	0.04
Exclusive breastfeeding until 3 months, no. (%)	30 049 (39.6)	1175	3031 (43.5)	95	0.03
Exclusive breastfeeding until 6 months, no. (%)	15 447 (20.3)	1175	1507 (21.7)	95	0.01
Neurodevelopmental delay at 6 months, no. (%)	6162 (8.4)	3769	559 (8.3)	322	0.00
Neurodevelopmental delay at 12 months, no. (%)	10 442 (14.6)	5381	888 (13.4)	443	0.01

[a] The difference between sibling samples versus the rest (n = 70 064). Effect sizes are calculated as *phi*/Cramer's *V* and *r*, using chi-square and Student's t tests for the categorical and numerical variables, respectively. SD, standard deviation; JPY, Japanese yen

Table 2. Association between any or exclusive BF and developmental delay for the full sample (n = 77 119)

	Developmental delay at 6 months			Developmental delay at 12 months		
	Number	cRR (95% CI)	aRR (95% CI) [a,b]	Number	cRR (95% CI)	aRR (95% CI) [a,b]
Any BF						
Until 6 months						
No	1263/12 967 (9.7%)	1 (reference)	1 (reference)	2091/12 735 (16.4%)	1 (reference)	1 (reference)
Yes	4899/60 383 (8.1%)	**0.83 (0.79 to 0.88)**	**0.81 (0.76 to 0.86)**	8351/59 003 (14.2%)	**0.86 (0.82 to 0.90)**	**0.81 (0.77 to 0.85)**
Until 12 months						
No	—	—	—	4061/25 303 (16.0%)	1 (reference)	1 (reference)
Yes	—	—	—	6381/46 435 (13.7%)	**0.86 (0.83 to 0.89)**	**0.81 (0.78 to 0.84)**

	Developmental delay at 6 months			Developmental delay at 12 months		
	Number	cRR (95% CI)	aRR (95% CI) [a]	Number	cRR (95% CI)	aRR (95% CI) [a]
Exclusive BF						
Until 3 months						
No	3794/43 558 (8.7%)	1 (reference)	1 (reference)	6637/42 648 (15.6%)	1 (reference)	1 (reference)
Yes	2273/28 686 (7.9%)	**0.91 (0.87 to 0.96)**	0.95 (0.90 to 1.00)	3664/28 051 (13.1%)	**0.84 (0.81 to 0.87)**	**0.86 (0.83 to 0.90)**
Until 6 months						
No	4768/57 508 (8.3%)	1 (reference)	1 (reference)	8228/56 374 (14.6%)	1 (reference)	1 (reference)
Yes	1299/14 736 (8.8%)	1.06 (1.00 to 1.13)	1.04 (0.98 to 1.11)	2073/14 325 (14.5%)	0.99 (0.95 to 1.04)	0.97 (0.92 to 1.01)

[a] Adjusted for sex, gestational age, birthweight, mother's age, maternal smoking and alcohol, maternal and paternal education, family income and home speech stimulation at 1 month. [b] Adjusted further for the introduction of complementary food. Boldface represents statistical significance ($P < 0.05$). Abbreviations: aRR, adjusted risk ratio; BF, breastfeeding; cRR, crude risk ratio; CI, confidence interval

Table 3. Association between duration of any or exclusive BF and total ASQ score for the full sample (n = 77 119)

	Increase of score at 6 months per BF month		Increase of score at 12 months per BF month	
	crude B (95% CI)	adjusted B (95% CI) [a,b]	crude B (95% CI)	adjusted B (95% CI) [a,b]
Duration of any BF (0 to 6 months)	**2.82 (2.20 to 3.61)**	**3.76 (2.89 to 4.88)**	**2.57 (1.94 to 3.40)**	**4.41 (3.27 to 5.95)**
Duration of any BF (0 to 12 months)	—	—	**1.60 (1.44 to 1.78)**	**2.15 (1.92 to 2.40)**

	Increase of score at 6 months per BF month		Increase of score at 12 months per BF month	
	crude B (95% CI)	adjusted B (95% CI) [a]	crude B (95% CI)	adjusted B (95% CI) [a]
Duration of exclusive BF (0 to 6 months)	**1.89 (1.66 to 2.14)**	**1.72 (1.51 to 1.95)**	**2.48 (2.15 to 2.87)**	**2.45 (2.12 to 2.84)**

[a] Adjusted for sex, gestational age, birthweight, mother's age, maternal smoking and alcohol, maternal and paternal education, family income and home speech stimulation at 1 month. [b] Adjusted further for the introduction of complementary food. Boldface represents statistical significance (*P* < 0.05). Abbreviations: ASQ, Ages and Stages Questionnaires; BF, breastfeeding; CI, confidence interval

Table 4. Selective analysis of sibling pairs discordant for any or exclusive BF among sibling sample (n = 7055)

	Developmental delay at 6 months				Developmental delay at 12 months			
	Number	Age diff., median (range)	cRR (95% CI)	aRR (95% CI)[a,b]	Number	Age diff., median (range)	cRR (95% CI)	aRR (95% CI)[a,b]
Any BF								
Until 6 months								
No	36/412 (8.7%)	22 m	1 (reference)	1 (reference)	65/414 (15.7%)	21 m	1 (reference)	1 (reference)
Yes	29/412 (7.0%)	(10 to 38 m)	0.80 (0.49 to 1.28)	0.65 (0.34 to 1.19)	55/414 (13.3%)	(10 to 38 m)	0.81 (0.54 to 1.17)	0.87 (0.55 to 1.34)
Until 12 months								
No			-	-	100/699 (14.3%)	22 m	1 (reference)	1 (reference)
Yes			-	-	78/699 (11.2%)	(10 to 38 m)	0.74 (0.54 to 1.01)	**0.64 (0.43 to 0.93)**

	Developmental delay at 6 months				Developmental delay at 12 months			
	Number	Age diff., median (range)	cRR (95% CI)	aRR (95% CI)[a]	Number	Age diff., median (range)	cRR (95% CI)	aRR (95% CI)[a]
Exclusive BF								
Until 3 months								
No	60/800 (7.5%)	24 m	1 (reference)	1 (reference)	96/755 (12.7%)	24 m	1 (reference)	1 (reference)
Yes	62/800 (7.8%)	(10 to 38 m)	1.04 (0.72 to 1.47)	0.95 (0.63 to 1.41)	97/755 (12.8%)	(10 to 39 m)	1.01 (0.74 to 1.37)	0.99 (0.69 to 1.38)
Until 6 months								
No	51/657 (7.8%)	24 m	1 (reference)	1 (reference)	70/633 (11.1%)	24 m	1 (reference)	1 (reference)
Yes	49/657 (7.5%)	(12 to 38 m)	0.95 (0.63–1.42)	0.77 (0.46 to 1.28)	83/633 (13.1%)	(12 to 38 m)	1.23 (0.88 to 1.69)	1.12 (0.74 to 1.65)

[a] Adjusted for sex, gestational age, birthweight, sibling order, maternal smoking and alcohol, and home speech stimulation at 1 month. [b] Adjusted further for the introduction of complementary food. Boldface represents statistical significance ($P < 0.05$). Abbreviations: Age diff., age difference between sibling pair; aRR, adjusted risk ratio; BF, breastfeeding; cRR, crude risk ratio; CI, confidence interval

Table 5. Association between duration of any or exclusive BF and total ASQ score for sibling sample (n = 7055)

	Increase of score per BF month	
	crude B (95% CI)	adjusted B (95% CI) [a,b]
ASQ age (6 *vs.* 12 months)	**12.8 (11.7 to 14.0)**	**12.9 (11.8 to 14.1)**
Duration of any BF (0 to 6 months)	**2.57 (1.38 to 3.75)**	**2.23 (1.05 to 3.41)**
ASQ age × duration of any BF	-0.57 (-1.69 to 0.55)	-0.40 (-1.53 to 0.73)

	Increase of score per BF month	
	crude B (95% CI)	adjusted B (95% CI) [a]
ASQ age (6 *vs.* 12 months)	**12.9 (11.7 to 14.0)**	**12.9 (11.8 to 14.1)**
Duration of exclusive BF (0 to 6 months)	1.00 (-0.15 to 2.15)	1.14 (-0.01 to 2.28)
ASQ age × duration of exclusive BF	0.65 (-0.48 to 1.77)	0.65 (-0.48 to 1.78)

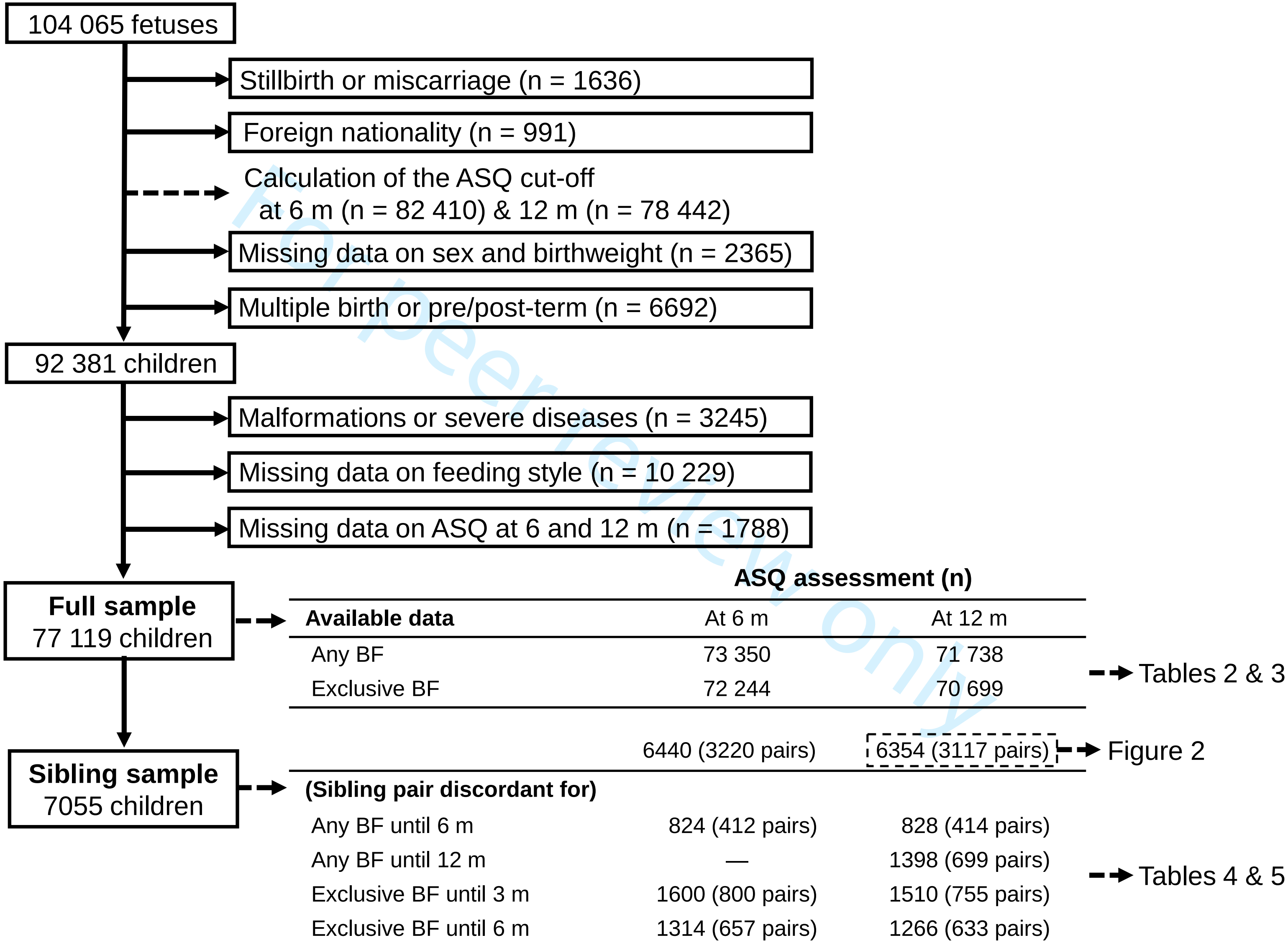
[a] Adjusted for sex, gestational age, birthweight, sibling order, maternal smoking and alcohol, and home speech stimulation at 1 month. [b] Adjusted further for the introduction of complementary food. Boldface represents statistical significance ($P < 0.05$).
Abbreviations: ASQ, Ages and Stages Questionnaires; BF, breastfeeding; CI, confidence interval

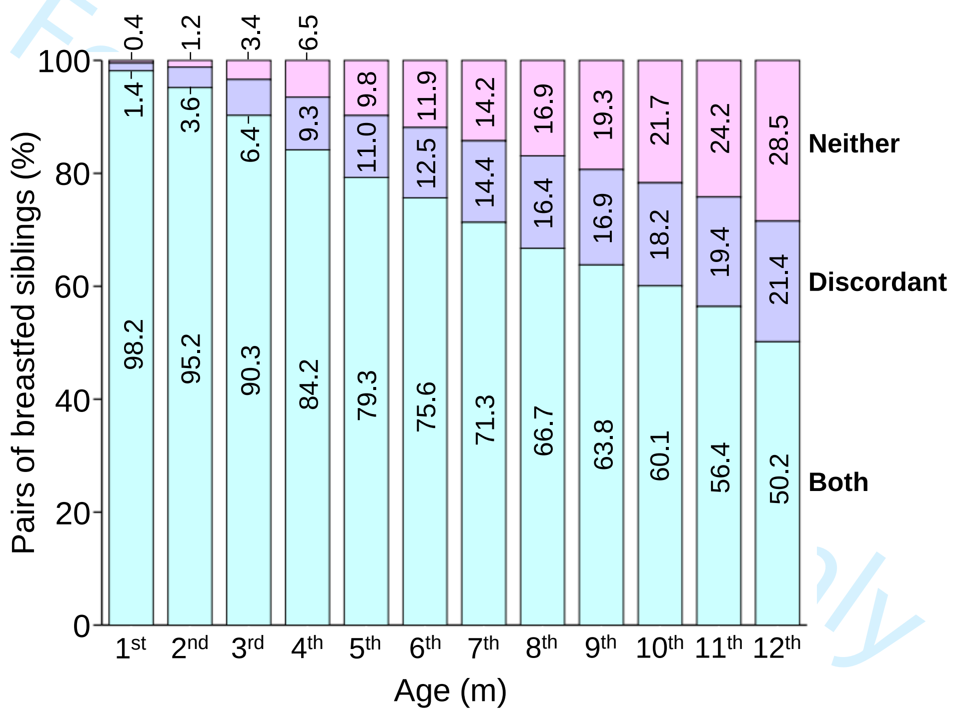
Figure Legends

Figure 1. Flowchart of participant selection.

ASQ, Ages and Stages Questionnaires; BF, breastfeeding

Figure 2. Pairs of siblings who were both breastfed, discordantly breastfed, or neither breastfed with respect to each month of life (n = 3117).


104 065 fetuses
Stillbirth or miscarriage (n = 1636)
Foreign nationality (n = 991)
Calculation of the ASQ cut-off at 6 m (n = 82 410) & 12 m (n = 78 442)
Missing data on sex and birthweight (n = 2365)
Multiple birth or pre/post-term (n = 6692)
92 381 children
Malformations or severe diseases (n = 3245)
Missing data on feeding style (n = 10 229)
Missing data on ASQ at 6 and 12 m (n = 1788)
Full sample 77 119 children
Sibling sample 7055 children
ASQ assessment (n)
Available data | At 6 m | At 12 m
Any BF | 73 350 | 71 738
Exclusive BF | 72 244 | 70 699
Tables 2 & 3
6440 (3220 pairs) | 6354 (3117 pairs)
Figure 2
(Sibling pair discordant for)
Any BF until 6 m | 824 (412 pairs) | 828 (414 pairs)
Any BF until 12 m | — | 1398 (699 pairs)
Exclusive BF until 3 m | 1600 (800 pairs) | 1510 (755 pairs)
Exclusive BF until 6 m | 1314 (657 pairs) | 1266 (633 pairs)
Tables 4 & 5


100
80
60
40
20
0
Pairs of breastfed siblings (%)
0.4
1.2
3.4
6.5
1.4
3.6
6.4
9.3
9.8
11.9
14.2
16.9
19.3
21.7
24.2
28.5
11.0
12.5
14.4
16.4
16.9
18.2
19.4
21.4
98.2
95.2
90.3
84.2
79.3
75.6
71.3
66.7
63.8
60.1
56.4
50.2
Neither
Discordant
Both
1st 2nd 3rd 4th 5th 6th 7th 8th 9th 10th 11th 12th
Age (m)

Supplementary Information on

Breastfeeding and infant development in a cohort with sibling pair analysis: the Japan Environment and Children's Study

Masafumi Sanefuji, Ayako Senju, Masayuki Shimono, Masanobu Ogawa, Yuri Sonoda, Michiko Torio, Yuko Ichimiya, Reiko Suga, Yasunari Sakai, Satoshi Honjo, Koichi Kusuhara, Shouichi Ohga, Japan Environment and Children's Study Group

Supplementary Figures

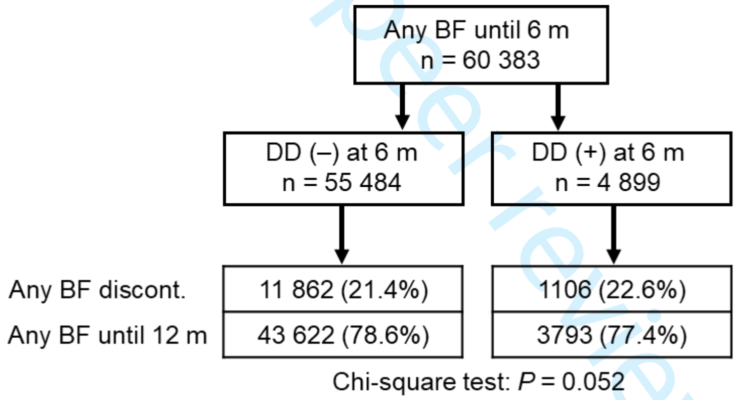


Figure S1. Continuation of any breastfeeding until 12 months in children with *vs.* without developmental delay at 6 months.
BF, breastfeeding; discont., discontinuation; DD, developmental delay

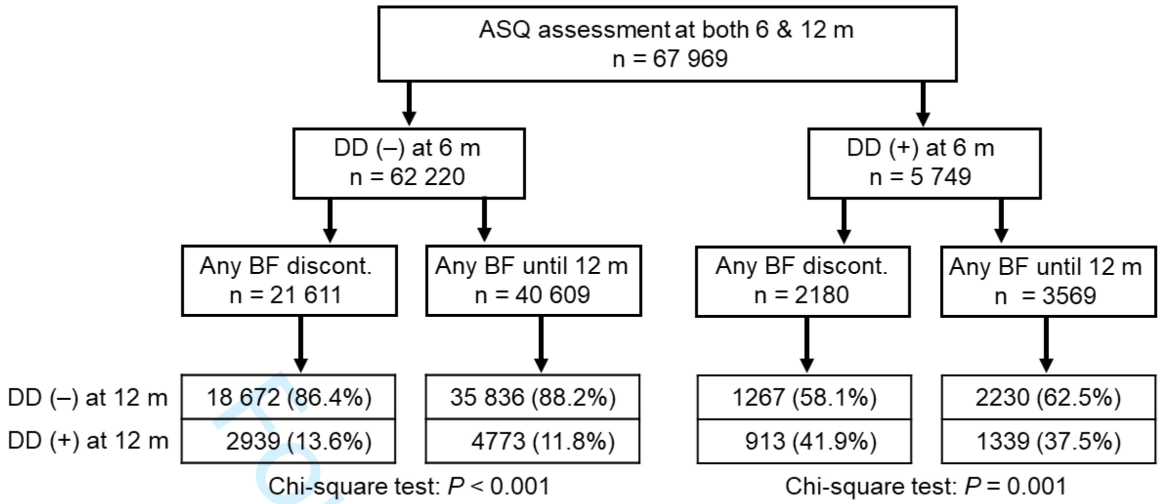


Figure S2. Developmental prognosis in children who continued any breastfeeding until 12 months *vs.* discontinued.
ASQ, Ages and Stages Questionnaires; BF, breastfeeding; discont., discontinuation; DD, developmental delay.

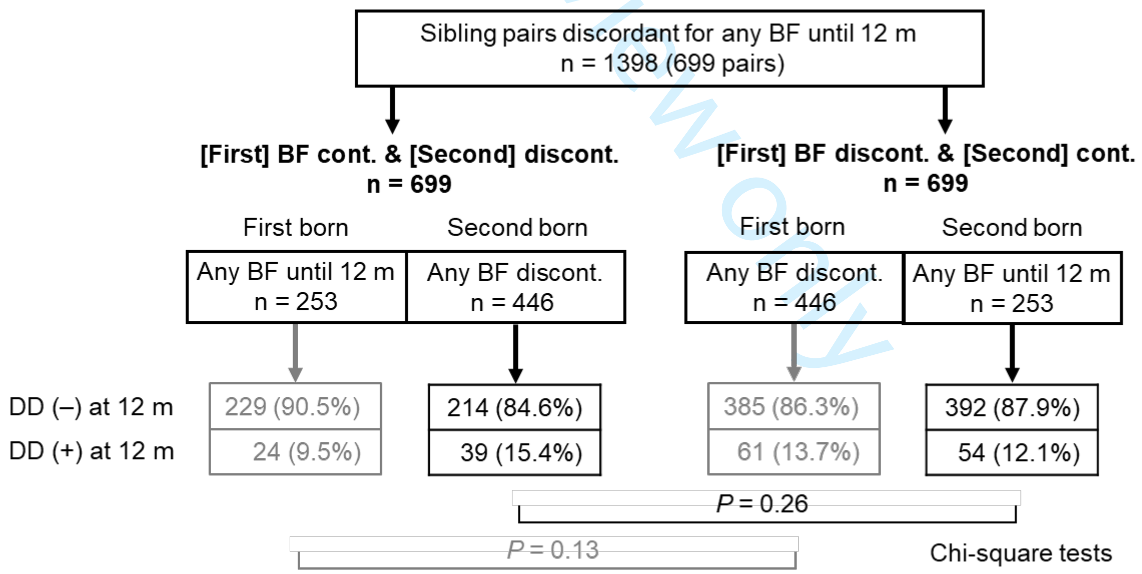


Figure S3. The risk of developmental delay of the second born sibling when the first born sibling continued any breastfeeding until 12 months *vs.* discontinued.
BF, breastfeeding; cont., continuation; discont., discontinuation; DD, developmental delay

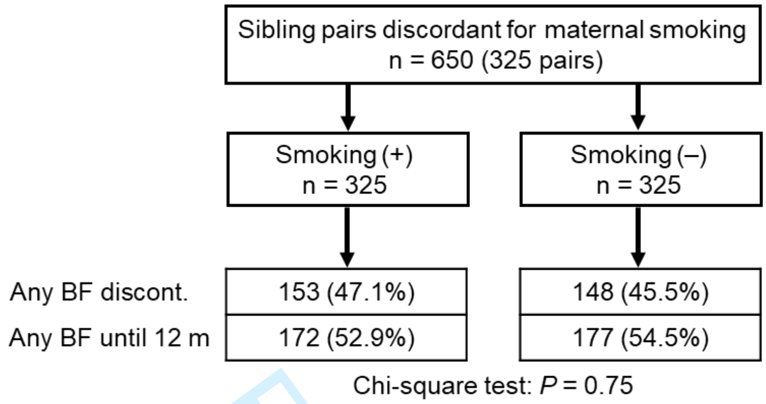


Figure S4. Continuation of any breastfeeding until 12 months in siblings whose mothers smoked during pregnancy *vs.* not.
BF, breastfeeding; discont., discontinuation

Supplementary Appendix

Members of the JECS Group as of 2020: Michihiro Kamijima (principal investigator; Nagoya City University, Nagoya, Japan), Shin Yamazaki (National Institute for Environmental Studies, Tsukuba, Japan), Yukihiro Ohya (National Center for Child Health and Development, Tokyo, Japan), Reiko Kishi (Hokkaido University, Sapporo, Japan), Nobuo Yaegashi (Tohoku University, Sendai, Japan), Koichi Hashimoto (Fukushima Medical University, Fukushima, Japan), Chisato Mori (Chiba University, Chiba, Japan), Shuichi Ito (Yokohama City University, Yokohama, Japan), Zentaro Yamagata (University of Yamanashi, Chuo, Japan), Hidekuni Inadera (University of Toyama, Toyama, Japan), Takeo Nakayama (Kyoto University, Kyoto, Japan), Hiroyasu Iso (Osaka University, Suita, Japan), Masayuki Shima (Hyogo College of Medicine, Nishinomiya, Japan), Youichi Kurozawa (Tottori University, Yonago, Japan), Narufumi Suganuma (Kochi University, Nankoku, Japan), Koichi Kusuhara (University of Occupational and Environmental Health, Kitakyushu, Japan), and Takahiko Katoh (Kumamoto University, Kumamoto, Japan).

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract Confirmed (page 3 of 30)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Confirmed (page 3)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Confirmed (pages 6 & 7)
Objectives	3	State specific objectives, including any prespecified hypotheses Confirmed (page 7)
Methods		
Study design	4	Present key elements of study design early in the paper Confirmed (page 7)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Confirmed (pages 7 & 8)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Confirmed (page 8)
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed Confirmed (page 11)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Confirmed (pages 9-11)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Confirmed (pages 8)
Bias	9	Describe any efforts to address potential sources of bias Confirmed (page 12, Table 1)
Study size	10	Explain how the study size was arrived at Confirmed (page 8)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Confirmed (pages 9 & 10)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding Confirmed (pages 11 & 12)
		(*b*) Describe any methods used to examine subgroups and interactions Confirmed (page 11)
		(*c*) Explain how missing data were addressed Confirmed (page 8, Figure 1, Table 1)
		(*d*) If applicable, explain how loss to follow-up was addressed Confirmed (page 8, Figure 1)
		(*e*) Describe any sensitivity analyses Not applicable

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Confirmed (pages 8 & 12, Figures 1-3, Table 1)
		(b) Give reasons for non-participation at each stage Confirmed (pages 13 & 14)
		(c) Consider use of a flow diagram Confirmed (Figure 1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Confirmed (Table 1)
		(b) Indicate number of participants with missing data for each variable of interest Confirmed (Table 1)
		(c) Summarise follow-up time (eg, average and total amount) Confirmed (page 8)
Outcome data	15*	Report numbers of outcome events or summary measures over time Confirmed (page 12, Table 1)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Confirmed (pages 13 & 14, Tables 2 & 3)
		(*b*) Report category boundaries when continuous variables were categorized Confirmed (pages 13)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Confirmed (pages 13 & 14)
Discussion		
Key results	18	Summarise key results with reference to study objectives Confirmed (page 14)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Confirmed (pages 17)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Confirmed (pages 15 & 16)
Generalisability	21	Discuss the generalisability (external validity) of the study results Confirmed (page 15)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based Confirmed (page 18)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Breastfeeding and infant development in a cohort with sibling pair analysis: the Japan Environment and Children's Study

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Secondary Subject Heading:	Paediatrics
Keywords:	EPIDEMIOLOGY, Developmental neurology & neurodisability < PAEDIATRICS, PAEDIATRICS

Breastfeeding and infant development in a cohort with sibling pair analysis: the Japan Environment and Children's Study

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ABSTRACT

Objectives

To investigate the association between breastfeeding and infant development during the first year of life using sibling comparison.

Design

Nationwide prospective birth cohort study with sibling pair analysis.

Setting

15 regional centres that participated in the Japan Environment and Children's Study.

Participants

This study included 77 119 children (singleton, term birth and no malformation/severe diseases) whose mothers were registered between January 2011 and March 2014, including 3 521 duos or trios of siblings.

Primary outcome measures

The primary outcome was developmental delay at 6 and 12 months of age, assessed using the Japanese translation of the Ages and Stages Questionnaires, third edition. Multivariable regression analyses adjusted for confounders were performed to estimate the risk ratios of delay associated with any or exclusive breastfeeding. Pairs of siblings discordant for statuses were selected, and conditional regression analyses were conducted with a matched cohort design.

Results

Developmental delay was identified in 6162 (8.4%) and 10 442 (14.6%) children at 6 and 12 months of age, respectively. Any breastfeeding continued until 6 months or 12 months old was associated with reduced developmental delay at 12 months of age (adjusted risk ratio [95% confidence interval]: 0.81 [0.77 to 0.85] and 0.81 [0.78 to 0.84], respectively). Furthermore, exclusive breastfeeding until 3 months was associated with reduced developmental delay at 12 months of age (0.86 [0.83 to 0.90]). In sibling pair analysis, the association between any breastfeeding until 12 months and reduced developmental delay at 12 months of age persisted (0.64 [0.43 to 0.93]).

Conclusions

The present study demonstrated the association of continuous breastfeeding with reduced developmental delay at 1 year of age using sibling pair analysis, in which unmeasured confounding factors are still present but less included. This may provide an argument to promote breastfeeding continuation.

Strengths and limitations of this study

- This study is the largest birth cohort study that investigated the association between breastfeeding and infant development.
- The association was examined using not only ordinary multivariable regression analysis but also sibling comparison, which strongly controls for sibling-shared factors.
- Monthly feeding status was collected at child's age of 1 month, 6 and 12 months, minimizing the risk of recall bias.
- Developmental delay was determined by a parent-reported screening test and thus may be equivocal.
- The results could not eliminate the possibility that the association still could be explained by reverse causation because the reason for cessation of breastfeeding was not known.

INTRODUCTION

Since 1929, the beneficial effects of breastfeeding on brain development have been repeatedly demonstrated.[1-4] Many observational studies[5-8] demonstrate that breastfeeding is associated with better cognitive outcomes, including neurodevelopment, language, and intelligence. In these studies, however, the causation remains unclear because the reason for cessation of breastfeeding is not known. Furthermore, this association can be produced by differences in demographic, socioeconomic, and environmental factors between mothers who breastfeed and those who do not.[9-12] In high-income countries, mothers with higher levels of education, social position, income, and intelligence are more inclined to breastfeed and to do so more exclusively and for a longer duration. Thus, their children are more likely to have higher cognitive functions, which can result in a superficial association between breastfeeding and better child cognition. In previous studies, the association disappeared or became highly diminished after controlling for confounders, especially maternal intelligence.[9 13 14] Nonetheless, a recent meta-analysis concluded that breastfeeding was significantly associated with higher cognitive abilities, even after adjusting for such confounding factors.[3]

After explicitly controlling for these measured factors, unmeasured—even unknown—confounders such as parental characteristics and child-rearing practices remained. To further control for these confounders, previous studies[9 15-17] conducted sibling pair analysis in investigating the association of breastfeeding with child cognitive outcomes. These analyses focused on siblings pairs who were discordant for breastfeeding exposure. A sibling pair from the same mother largely shares parental and environmental factors. Thus, the effects of these confounders can be cancelled out when the pair is matched in the analysis. However, on this topic, sibling pair analysis is challenging because little variation in breastfeeding often exists between siblings, which may reduce statistical power and erroneously cause null findings.[17] To our knowledge, only three studies[9 15 16] have examined the association between breastfeeding and cognitive functions using this method, and these studies all produced statistically null effects. The reason for the null results remains unclear. However, these findings may be accounted for by the study designs: data on feeding status were collected only once within 1 year[9] or 2 years[15] after a child's birth or in adolescence.[16]

The goal of the current study was to investigate the association between breastfeeding and child development during the first year of life by using data from the Japan Environment and Children's Study (JECS). This nationwide birth cohort study includes >100,000 children and thus enables sibling pair analysis with a sufficient number of participants. The monthly status of breastfeeding was collected repeatedly in the first year of life, thereby minimizing the risk of recall bias. The beneficial effects of breastfeeding on cognitive development decrease as children age;[18] therefore, investigating the association between breastfeeding and cognitive development during early childhood has the advantage of allowing researchers to infer the role of breastfeeding on the developing brain.

METHODS

Design

The JECS is a nationwide, multicenter, prospective birth cohort study funded by the Ministry of Environment, Japan. The details of the study design have been described elsewhere.[19 20] Briefly, pregnant participants were registered between January 2011 and March 2014 in 15 regional centers covering a wide geographical area in Japan. During pregnancy, data on demographics, smoking, alcohol, education, and socioeconomic statuses were obtained during the first and second/third trimesters by using self-administered questionnaires. Detailed information regarding the mother and child was obtained from medical records transcripts during the first trimester, at the time of delivery, and when the child was 1 month old. After delivery, data on feeding style, use of complementary foods, developmental status, and affected diseases were collected at ages 1 and 6 months and every 6 months until the child was 6 years old, and then twice a year thereafter *via* self-reported questionnaires completed by the parents.

The JECS protocol was reviewed and approved by the Ministry of Environment's Institutional Review Board for Epidemiological Studies and by the ethics committees of all participating institutions (No.100910001). The ethical approval for this study was an extension of the ethical approval for the JECS protocol. Written informed consent was obtained from all parents.

Participants

In this study, we used the fixed dataset "jecs-an-20180131" that was released in March 2018. This dataset contains all available data extracted from the aforementioned questionnaires and records until a child was 12 months old. The data for 104 065 fetuses from 103 062 pregnancies were linked to the respective maternal data. The participants selected were 92 381 live-born singleton children, delivered at term (≥37 gestational weeks and <42 gestational weeks), of parents of Japanese nationality and for whom information on sex and birthweight had been recorded (Figure 1). Of these children, those who had malformations or severe diseases, or who had missing information on feeding style during the first year of life or development at 6 months and 12 months old were excluded. After these exclusions, the data of 77 119 children were included in our analysis.

Exposure

The main exposure factor was breastfeeding. Mothers were asked to fill in the monthly feeding status of their child by using questionnaires when the child was 1 month, 6 months, and 12 months old. This information included whether the child was breastfed, formula-fed, or both. The questionnaire administered when the child was 12 months old also queried when complementary food was first started. Breastfeeding duration indicated how long a child was breastfed from birth, irrespective of concurrent consumption of formula milk. We also dichotomously assessed whether or not a child continued (1) any breastfeeding until 6 months old, (2) any breastfeeding until 12 months, (3) exclusive breastfeeding until 3 months, and (4) exclusive breastfeeding until 6 months. Breastfeeding was "exclusive" if the child consumed only breastmilk—and nothing else (no consumption of formula milk or complementary foods) during these periods. To gain more insight into the significance of exclusive breastfeeding, we further classified the children who continued breastfeeding until 6 months into four categories:

(1) children who ingested neither formula milk nor complementary food (exclusive breastfeeding), (2) those who ingested formula but not complementary food, (3) those who ingested complementary food but not formula, and (4) those who ingested both formula and complementary food, at any time during the period.

For sibling pair analysis, we selected pairs who were discordant on the status of any breastfeeding or exclusive breastfeeding. When discordance was observed among three siblings (e.g., only one of the three children was breastfed), we randomly selected one of the two siblings who were not breastfed and then paired the selected one with the breastfed sibling.

Outcome

The outcome was developmental delay measured at 6 months and 12 months old, using the Japanese translated version of the Ages and Stages Questionnaires: A Parent-Completed Monitoring System (ASQ), third edition. This version was prepared through a back-translation procedure and was approved by the publisher of the original English version.[21] The ASQ can identify infants or young children who need further developmental assessment to determine whether they are eligible for early intervention. The findings of the questionnaire basically agree with those of professionally administered developmental batteries.[22 23] It has been used in clinical and research settings and translated into several languages.[24-27] The ASQ assesses five developmental domains. For each domain, six skills are described to which parents answer "yes," "sometimes," or "not yet," depending on whether their child is demonstrating the described skill. The responses are converted to points, with "yes" receiving 10 points;

“sometimes”, 5 points; and “not yet”, 0 points. The child’s score for each developmental domain is the sum of all points received for the items under that domain and ranges from 0 to 60 points. The cut-off score for each domain was defined as two standard deviations below the mean score of large standardized samples in the United States of America. A child was defined as having a developmental delay if a score was at or below the cut-off level in any developmental domain. When the cut-off scores of the original English version were used in our population, an excessive number of children were classified as having a developmental delay (47.4% and 34.6% for 6 months and 12 months, respectively). Although preliminary cut-off scores of the Japanese translation were recently proposed,[28] these were not recommended to be used with confidence before 24 months old because of very limited sample sizes. Therefore, the cut-off scores were determined by using the same methodologies used in the original version, based on available data at ages 6 months (n = 82 410) and 12 months (n = 78 442) (Figure 1), which would represent the general Japanese population. As a continuous variable, in addition, total score of ASQ was defined as the sum of the scores for the five domains, ranging from 0 to 300 points.

Statistical analysis

To assess the association of breastfeeding with child development, we conducted multivariable quasi-Poisson regression analyses for dichotomous dependent variables, and multiple linear regression analyses for continuous dependent variables. The adjusted covariates were i) sex, ii) gestational age, iii) birthweight, iv) mother’s age, v) maternal smoking status during pregnancy, as recorded in the first trimester, vi) maternal alcohol consumption during pregnancy, as recorded in the second trimester, vii) maternal and viii) paternal education level (junior high school, high school, and university or graduate school), ix) annual family income (<4 000 000; 4 000 000–5 999 999; ≥6 000 000 JPY), x) introduction of complementary foods before 6 months old, and xi) home speech stimulation at 1 month (whether a mother did or did not talk to her baby habitually: yes/no). The "home speech stimulation" covariate was used instead of the Home Observation for Measurement of the Environment scale,[29] which is not employed in the JECS.

For sibling pair analysis, we conducted conditional logistic regression analyses with 1:1 matched cohort data of sibling pairs whose dichotomous statuses of breastfeeding were discordant.[30] We reported adjusted relative risks (aRRs) with 95% confidence intervals (CIs) that were converted from odds ratios using an established method.[31 32] We also used a longitudinal linear mixed model, in which fixed effects were age of ASQ assessment (6 *vs.* 12 months old), duration of breastfeeding, and the interaction term between them, with random intercept for sibling. The adjusted covariates were as follows: i) sex, ii) gestational age, iii) birthweight, iv) order of siblings in the discordant pair, v) maternal smoking status, vi) maternal alcohol consumption, vii) complementary food introduction, and viii) home speech stimulation at 1 month old. All statistical analyses were conducted using R software (version 3.5.0). In the R package, we used "survival" (version 3.2.7) for conditional logistic regression model and "lme4" for longitudinal linear mixed model. The level of significance was $P = 0.05$.

Patient and public involvement

No participants were involved in creating the research question or the outcome measures, nor were they involved in developing plans for recruitment, design or implementation of the study. No participants were asked to provide advice on the interpretation or writing up of the results. There are plans to disseminate the results of the research to study participants and the general public. Participants were thanked in the acknowledgments.

RESULTS

The baseline characteristics of 77 119 children are summarized in Table 1. Nearly all (76 167, 98.8%) children were started on any breastmilk during their first month of life. Any breastfeeding was continued until ages 6 and 12 months in 82.1% and 64.4% of children, respectively. Exclusive breastfeeding was continued until ages 3 and 6 months in 39.6% and 20.3% of children, respectively. Developmental delay was identified in 8.4% and 14.6% of children at 6 months and 12 months old, respectively. The sibling cohort included 3521 sibling sets (7055 children) in total: 3508 duos (7016 children) and 13 trios (39 children). The characteristics of the sibling sample were substantially similar to those of the full sample. Nevertheless, the sibling sample appeared to have weak tendencies towards younger maternal age, lower paternal education, lower family income, lower rates for any breastfeeding until 12 months old, and higher rates for exclusive breastfeeding until 3 months.

For the full sample (n = 77 119), we conducted multivariable regression analyses, while adjusting for confounders, to examine developmental delay in relation to various types of breastfeeding exposures. When breastfeeding was treated as dichotomous variables, quasi-Poisson models revealed that any breastfeeding continued until 6 months was associated with reduced developmental delay at ages 6 months (aRR [95% CI]: 0.81 [0.76 to 0.86]) and 12 months (0.81 [0.77 to 0.85]) (Table 2a). Any breastfeeding until 12 months was similarly associated with reduced developmental delay at age 12 months (0.81 [0.78 to 0.84]). Any breastfeeding was similarly continued until 12 months old between children with (77.4%) and without developmental delay (78.6%) at 6 months old (Figure S1), arguing against the possibility that developmental delay *per se* interrupted the continuation of breastfeeding. When developmental delay was not observed at 6 months old, it is more likely to occur newly at 12 months in children who discontinued breastfeeding by 12 months old than those continued it while delay at 6 months resolved more often in children who continued breastfeeding (Figure S2). Furthermore, exclusive breastfeeding that continued until 3 months old, but not until 6 months, was associated with developmental delay at age 12 months (0.86 [0.83 to 0.90]), Table 2b). Among the children who continued breastfeeding until 6 months old and had the information on complementary food, the effects of formula milk and complementary food was estimated, referenced to exclusive breastfeeding. The risk of developmental delay at 6 months was reduced in children who concomitantly ingested complementary food, irrespective of formula feeding. The risk of developmental delay at 12 months was increased in those who concurrently ingested formula milk without complementary food, but was reduced in those who ingested complementary food with no formula (Table 2c). When breastfeeding duration was treated as a continuous variable, multiple linear regression model demonstrated that duration of any or exclusive breastfeeding was positively associated with increased total ASQ scores at 6 and 12 months old (Table 3).

To conduct sibling pair analysis, we extracted data from pairs of siblings who both underwent a developmental assessment at 6 months old (3220 pairs) and 12 months old (3117 pairs). Among these children, we further selected sibling pairs who were discordant for various breastfeeding statuses (Figure 1 and Table 4). Few variations existed in the statuses between pairs; therefore, the number of selected pairs was relatively small, varying from 412 pairs (824 children) to 800 pairs (1600 children), based on age (3 months, 6 months, or 12 months) and type (any breastfeeding or exclusive breastfeeding). Among these combinations, the adjusted conditional regression model for 699 sibling pairs (1398 children) revealed that any breastfeeding until 12 months was significantly associated with reduced developmental delay at this age (0.64 [0.43 to 0.93]). The mean breastfeeding duration was 12 months in the sibling who was continuously breastfed and 7.8 ± 2.9 months in the sibling who was not. Moreover, exclusive breastfeeding was not significantly associated with reduced developmental delay at any age. In sibling pairs discordant for any breastfeeding until 12 months, when the first-born children continued breastfeeding, the second-born, who discontinued it, had a tendency for developmental delay at 12 months; when the first born discontinued breastfeeding, the second showed a reduced tendency (Figure S3). In sibling pairs who were discordant for maternal smoking, a proxy for socioeconomical status at that time, any breastfeeding was similarly continued until 12 months old between children whose mothers had smoking (52.9%) *vs.* no smoking (54.5%) during pregnancy (Figure S4). When breastfeeding was treated as continuous variables, longitudinal linear mixed model revealed that duration of any, but not exclusive, breastfeeding was associated with increased total ASQ score (Table 5).

To clarify how differently siblings were breastfed during the first year of life, we classified 3117 pairs whose developmental assessment at 12 months old was recorded into 3 groups: “both” (both children were breastfed), “discordant” (only one child was breastfed), and “neither” (neither child was breastfed) (Figure 2). The number of discordant pairs increased from 43 (1.4%) pairs at the first month of life to 389 (12.5%) pairs at 6 months and 666 (21.4%) pairs at 12 months.

DISCUSSION

The present study investigated the relationship between breastfeeding and child development during the first year of life. Ordinary multivariable regression analyses demonstrated that any breastfeeding continued until 6 or 12 months old, and exclusive breastfeeding until 3 months were significantly associated with reduced developmental delay. In the sibling pair analysis, only the association between any breastfeeding until 12 months old and reduced developmental delay at 12 months old remained significant. The null association of any breastfeeding until 6 months might be explained by failure to detect less developmental variations at 6 months compared with those at 12 months, or involvement of other environmental factors that child had experienced after 6 months old.

The association that we observed between breastfeeding and brain functions has repeatedly been reported in observational, meta-analysis, and randomized controlled studies.[3 4]

[7 8 33] In these studies, the results were heterogeneously adjusted for various parental and environmental confounders. However, no matter how many measured confounders are included, unmeasured confounding factors always exist. Hence, we opted for sibling pair analysis, which controls for all factors shared by siblings from the same mother.[17] We observed a significant association between breastfeeding and development at 12 months old. Our findings further support the World Health Organization's recommendations concerning continued breastfeeding beyond 6 months old.[2] The reason for our significant results is unlikely to be explained simply by the sufficient number of our discordant pairs of siblings (1398 children), which is comparable to the number in previous studies[9 15 16] reporting null findings (1046, 1090, and 1773 children). A possible explanation is that we assessed child's development in the first year of life, whereas the previous studies assessed it at 4–14 years old. A randomized control study showed that the beneficial effects of breastmilk on cognitive development decrease with advancing age; thus, other environmental and genetic factors may become more important as children age.[18]

The mechanisms underlying the association between breastfeeding and brain development are unclear but may be attributable to its nutrients such as long-chain polyunsaturated fatty acids, hormones and cytokines.[34 35] Another probable mechanism is mother-infant interaction produced by breastfeeding behaviors.[36] A series of Family Nurture Intervention study have repeatedly demonstrated the importance of early nurturing activities that engage the mother and infant reciprocally in physical, sensory, and emotional experiences in infant development.[37-43] Such nurturing activities *via* breastfeeding may enhance the connection between social motivation and mother-infant relational health,[44] leading to better development.

In contrast to any breastfeeding, exclusive breastfeeding had no significant association with developmental delay in our study. Research on the association between exclusive breastfeeding and cognitive development is relatively scarce and has yielded inconsistent results: some studies report positive effects of exclusive breastfeeding on neurodevelopment,[45-46] whereas other studies report limited or rather negative effects.[47-49] The reason for the reduced effects of exclusive breastfeeding versus that of any breastfeeding is not well understood. Our results showed that concomitant ingestion of complementary food, but not formula milk, was associated with reduced developmental delay in the children who continued breastfeeding until 6 months old (Table 2c). Thus, breastmilk without supplementation of complementary food may not meet the full requirements for energy and micronutrients such as iron and zinc, which all have important roles in the developing brain,[50] of the average infant at 6 months old, as some researchers suggested.[51] Withholding complementary food until age 6 months may negate the beneficial effects of breastfeeding. Alternatively, such withholding might reflect some unmeasured confounders that adversely related to infant development.

In this study, the number of pairs who were discordantly breastfed in the first year of life increased with age, with the least discordance being at 1 month old, at which point 98.2% of the sibling pairs were both breastfed. This finding suggests that most mothers breastfeed their children in early infancy but discontinue later at different times for each sibling. Thus, the association between breastfeeding and development is likely related more to breastfeeding late into year 1 rather than breastfeeding early. By contrast, a previous randomized controlled trial[33] in which participants were randomly assigned to a breastfeeding promotion intervention group demonstrated that discordance in breastfeeding between an intervention group and control group was larger in early infancy than later in the first year of life. Late discordance such as that in the present study may be common in studies with an observational design. The brain is more sensitive to environmental factors earlier in life; therefore, the discordance later in life may produce less divergent impacts on brain development between siblings. This factor may explain, at least partially, the null results of sibling comparison in previous observational studies.[9 15 16]

Strengths and limitations

To our knowledge, this study is the largest birth cohort study examining the association between breastfeeding and brain function. We conducted sibling pair analyses with a sufficient number of participants from this large cohort, which enabled us to have strong control over sibling-shared parental and environmental factors. Monthly information on feeding methods was precisely obtained *via* successive questionnaires at 1 month, 6 months, and 12 months old, which yielded a much smaller risk of recall bias than that of previous sibling pair studies.[9 15 16]

The current study does have several limitations. The information was largely obtained from self-administered questionnaires. In particular, the identified developmental delay may be somewhat equivocal because it relied solely on responses on the parent-reported screening test of Japanese version of ASQ. Furthermore, even in sibling pair analysis, other confounding factors such as environmental factors may be responsible for the differences because siblings do not share all environmental factors and shared environments may not always be stable.[17] Finally, there were no data on what factors have contributed to cessation of breastfeeding. Even within a pair of sibling, there could be difference in socioeconomical status, which might alter parent's rearing behaviors and then affect the child's development. If an infant at potential risk of developmental disorders has less preference to breastfeeding, a superficial association can be produced between breastfeeding and better development. Indeed, a meta-analysis demonstrated altered feeding habits in children with attention-deficit/hyperactivity disorder.[52] Although our supplementary analyses rather argued against such possibility, the association between breastfeeding and a reduced risk of developmental delay in our study still could be explained by such reverse causation.

CONCLUSION

The present study demonstrated for the first time, by using sibling pair analysis, an association of continuous breastfeeding with reduced developmental delay at 1 year old. Although causal inference should be cautious in observational studies, both the prospective longitudinal and family-based matched analyses presented may provide a more persuasive argument for public health practitioners and policymakers to promote breastfeeding continuation, at least during the first year of life. The ongoing JECS cohort may reveal how long the observed beneficial effects will persist in later life.

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Contributors

M. Sanefuji conceived, designed the study, analysed the data, interpreted the results and wrote the manuscript. AS, M. Shimono and MO interpreted the results and critically reviewed the manuscript. Y. Sonoda, MT, YI, RS and Y. Sakai critically reviewed the manuscript. SH analysed the data, interpreted the results and critically reviewed the manuscript. KK and SO directed the study and critically reviewed the manuscript.

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Data sharing

No data are available. For details, see http://www.env.go.jp/chemi/ceh/en/

Ethics Approval

The JECS protocol was reviewed and approved by the Ministry of Environment's Institutional Review Board for Epidemiological Studies and by the ethics committees of all participating institutions (No.100910001). The ethical approval for this study was an extension of the ethical approval for the JECS protocol. Written informed consent was obtained from all parents.

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Table 1. Baseline characteristics of the children

	Full sample (n = 77 119)	Missing	Sibling sample (n = 7055)	Missing	Effect size [a]
Boy, no. (%)	39 350 (51.0)	0	3552 (50.3)	0	0.00
Gestational age (wk.), mean (SD)	39.5 (1.1)	0	39.5 (1.1)	0	0.00
Birth weight (g), mean (SD)	3062 (365)	0	3079 (360)	0	0.01
Maternal age (y), mean (SD)	31.3 (4.9)	4	29.8 (4.6)	0	0.09
Maternal smoking during pregnancy, no. (%)	12 424 (16.3)	858	1062 (15.2)	58	0.01
Maternal alcohol during pregnancy, no. (%)	2080 (2.7)	875	231 (3.3)	71	0.01
Maternal education, no. (%)		700		49	0.02
Junior high school	3029 (4.0)		310 (4.4)		
High school	56 180 (73.5)		5264 (75.1)		
University/graduate school	17 210 (22.5)		1432 (20.4)		
Paternal education, no. (%)		1111		62	0.03
Junior high school	4960 (6.5)		541 (7.7)		
High school	44 973 (59.2)		4381 (62.6)		
University/graduate school	26 075 (34.3)		2071 (29.6)		
Family income, no. (%)		5454		427	0.03
Low (<4,000,000 JPY)	28 012 (39.1)		2836 (42.8)		
Middle (4,000,000–5,999,999 JPY)	24 070 (33.6)		2189 (33.0)		
High (≥6,000,000 JPY)	19 583 (27.3)		1603 (24.2)		
Complementary food before 6 months, no. (%)	34 126 (44.9)	1175	3194 (45.9)	95	0.01
Home speech stimulation at 1 month, no. (%)	62 400 (81.1)	214	5611 (79.7)	17	0.01
Any breastfeeding until 1 month, no. (%)	76 167 (98.8)	0	6976 (98.9)	0	0.00
Any breastfeeding until 6 months, no. (%)	63 296 (82.1)	0	5713 (81.0)	0	0.01
Any breastfeeding until 12 months, no. (%)	49 672 (64.4)	0	4148 (58.8)	0	0.04
Exclusive breastfeeding until 3 months, no. (%)	30 049 (39.6)	1175	3031 (43.5)	95	0.03
Exclusive breastfeeding until 6 months, no. (%)	15 447 (20.3)	1175	1507 (21.7)	95	0.01
Neurodevelopmental delay at 6 months, no. (%)	6162 (8.4)	3769	559 (8.3)	322	0.00
Neurodevelopmental delay at 12 months, no. (%)	10 442 (14.6)	5381	888 (13.4)	443	0.01

[a] The difference between sibling samples versus the rest (n = 70 064). Effect sizes are calculated as *phi*/Cramer's *V* and *r*, using chi-square and Student's t tests for the categorical and numerical variables, respectively. JPY, Japanese yen; SD, standard deviation

Table 2. Association between developmental delay and any or exclusive BF for the full sample (n = 77 119)

	Developmental delay at 6 months			Developmental delay at 12 months		
	Number	cRR [95% CI]	aRR [95% CI] [a,b]	Number	cRR [95% CI]	aRR [95% CI] [a,b]
a. Any BF						
Until 6 months						
No	1263/12 967 (9.7%)	1 [reference]	1 [reference]	2091/12 735 (16.4%)	1 [reference]	1 [reference]
Yes	4899/60 383 (8.1%)	**0.83 [0.79 to 0.88]**	**0.81 [0.76 to 0.86]**	8351/59 003 (14.2%)	**0.86 [0.82 to 0.90]**	**0.81 [0.77 to 0.85]**
Until 12 months						
No	—	—	—	4061/25 303 (16.0%)	1 [reference]	1 [reference]
Yes	—	—	—	6381/46 435 (13.7%)	**0.86 [0.83 to 0.89]**	**0.81 [0.78 to 0.84]**

	Developmental delay at 6 months			Developmental delay at 12 months		
	Number	cRR [95% CI]	aRR [95% CI] [a]	Number	cRR (95% CI)	aRR (95% CI) [a]
b. Exclusive BF						
Until 3 months						
No	3794/43 558 (8.7%)	1 [reference]	1 [reference]	6637/42 648 (15.6%)	1 [reference]	1 [reference]
Yes	2273/28 685 (7.9%)	**0.91 [0.87 to 0.96]**	0.95 [0.90 to 1.00]	3664/28 050 (13.1%)	**0.84 [0.81 to 0.87]**	**0.86 [0.83 to 0.90]**
Until 6 months						
No	4768/57 508 (8.3%)	1 [reference]	1 [reference]	8228/56 374 (14.6%)	1 [reference]	1 [reference]
Yes	1299/14 735 (8.8%)	1.06 [1.00 to 1.13]	1.04 [0.98 to 1.11]	2073/14 324 (14.5%)	0.99 [0.95 to 1.04]	0.97 [0.92 to 1.01]

	Developmental delay at 6 months			Developmental delay at 12 months		
	Number	cRR [95% CI]	aRR [95% CI] [a]	Number	cRR [95% CI]	aRR [95% CI] [a]
c. BF until 6 months						
FF(−), CF(−) (= exclusive BF)	1299/14 735 (8.8%)	1 [reference]	1 [reference]	2073/14 324 (14.5%)	1 [reference]	1 [reference]
FF(+), CF(−)	1713/18 482 (9.3%)	1.05 [0.98 to 1.13]	1.01 [0.94 to 1.08]	2935/17 985 (16.3%)	**1.13 [1.07 to 1.19]**	**1.09 [1.03 to 1.15]**
FF(−), CF(+)	631/9960 (6.3%)	**0.72 [0.66 to 0.79]**	**0.79 [0.72 to 0.87]**	1087/9857 (11.0%)	**0.76 [0.71 to 0.82]**	**0.82 [0.77 to 0.88]**
FF(+), CF(+)	1184/16 314 (7.3%)	**0.82 [0.76 to 0.89]**	**0.87 [0.81 to 0.95]**	2145/16 000 (13.4%)	**0.93 [0.88 to 0.98]**	0.97 [0.91 to 1.03]

[a] Adjusted for sex, gestational age, birthweight, mother's age, maternal smoking and alcohol, maternal and paternal education, family income and home speech stimulation at 1 month. [b] Adjusted further for the introduction of complementary food. Boldface represents statistical significance ($P < 0.05$). Abbreviations: aRR, adjusted risk ratio; BF, breastfeeding; CF, complementary food; CI, confidence interval; cRR, crude risk ratio; FF, formula feeding

Table 3. Association between duration of any or exclusive BF and total ASQ score for the full sample (n = 77 119)

	Increase of score at 6 months per BF month		Increase of score at 12 months per BF month	
	crude B [95% CI]	adjusted B [95% CI] [a,b]	crude B [95% CI]	adjusted B [95% CI] [a,b]
Duration of any BF (0 to 6 months)	**1.04 [0.79 to 1.28]**	**1.32 [1.06 to 1.58]**	**0.94 [0.66 to 1.22]**	**1.48 [1.18 to 1.78]**
Duration of any BF (0 to 12 months)	—	—	**0.47 [0.36 to 0.57]**	**0.77 [0.65 to 0.88]**

	Increase of score at 6 months per BF month		Increase of score at 12 months per BF month	
	crude B [95% CI]	adjusted B [95% CI] [a]	crude B [95% CI]	adjusted B [95% CI] [a]
Duration of exclusive BF (0 to 6 months)	**0.63 [0.51 to 0.76]**	**0.54 [0.41 to 0.67]**	**0.91 [0.77 to 1.05]**	**0.90 [0.75 to 1.04]**

[a] Adjusted for sex, gestational age, birthweight, mother's age, maternal smoking and alcohol, maternal and paternal education, family income and home speech stimulation at 1 month. [b] Adjusted further for the introduction of complementary food. Boldface represents statistical significance ($P < 0.05$). Abbreviations: ASQ, Ages and Stages Questionnaires; BF, breastfeeding; CI, confidence interval

Table 4. Selective analysis of sibling pairs discordant for any or exclusive BF among sibling sample (n = 7055)

	Developmental delay at 6 months				Developmental delay at 12 months			
	Number	Age diff., median (range)	cRR [95% CI]	aRR [95% CI] [a,b]	Number	Age diff., median (range)	cRR [95% CI]	aRR [95% CI] [a,b]
Any BF								
Until 6 months								
No	36/412 (8.7%)	22 m	1 [reference]	1 [reference]	65/414 (15.7%)	21 m	1 [reference]	1 [reference]
Yes	29/412 (7.0%)	(10 to 38 m)	0.80 [0.49 to 1.28]	0.65 [0.34 to 1.19]	55/414 (13.3%)	(10 to 38 m)	0.81 [0.54 to 1.17]	0.87 [0.55 to 1.34]
Until 12 months								
No			-	-	100/699 (14.3%)	22 m	1 [reference]	1 [reference]
Yes			-	-	78/699 (11.2%)	(10 to 38 m)	0.74 [0.54 to 1.01]	**0.64 [0.43 to 0.93]**

	Developmental delay at 6 months				Developmental delay at 12 months			
	Number	Age diff., median (range)	cRR [95% CI]	aRR [95% CI] [a]	Number	Age diff., median (range)	cRR [95% CI]	aRR [95% CI] [a]
Exclusive BF								
Until 3 months								
No	60/800 (7.5%)	24 m	1 [reference]	1 [reference]	96/755 (12.7%)	24 m	1 [reference]	1 [reference]
Yes	62/800 (7.8%)	(10 to 38 m)	1.04 [0.72 to 1.47]	0.95 [0.63 to 1.41]	97/755 (12.8%)	(10 to 39 m)	1.01 [0.74 to 1.37]	0.99 [0.69 to 1.38]
Until 6 months								
No	51/657 (7.8%)	24 m	1 [reference]	1 [reference]	70/633 (11.1%)	24 m	1 [reference]	1 [reference]
Yes	49/657 (7.5%)	(12 to 38 m)	0.95 [0.63–1.42]	0.77 [0.46 to 1.28]	83/633 (13.1%)	(12 to 38 m)	1.23 [0.88 to 1.69]	1.12 [0.74 to 1.65]

[a] Adjusted for sex, gestational age, birthweight, sibling order, maternal smoking and alcohol, and home speech stimulation at 1 month. [b] Adjusted further for the introduction of complementary food. Boldface represents statistical significance (*P* < 0.05). Abbreviations: Age diff., age difference between sibling pair; aRR, adjusted risk ratio; BF, breastfeeding; CI, confidence interval; cRR, crude risk ratio

Table 5. Association between duration of any or exclusive BF and total ASQ score for sibling sample (n = 7055)

	Increase of score per BF month	
	crude B [95% CI]	adjusted B [95% CI] [a,b]
ASQ age (6 months [0] *vs.* 12 months [1])	**12.8 [11.7 to 14.0]**	**12.9 [11.8 to 14.1]**
Duration of any BF (0 to 6 months)	**2.57 [1.38 to 3.75]**	**2.23 [1.05 to 3.41]**
ASQ age × duration of any BF	-0.57 [-1.69 to 0.55]	-0.40 [-1.53 to 0.73]

	Increase of score per BF month	
	crude B [95% CI]	adjusted B [95% CI] [a]
ASQ age (6 months [0] *vs.* 12 months [1])	**12.9 [11.7 to 14.0]**	**12.9 [11.8 to 14.1]**
Duration of exclusive BF (0 to 6 months)	1.00 [-0.15 to 2.15]	1.14 [-0.01 to 2.28]
ASQ age × duration of exclusive BF	0.65 [-0.48 to 1.77]	0.65 [-0.48 to 1.78]

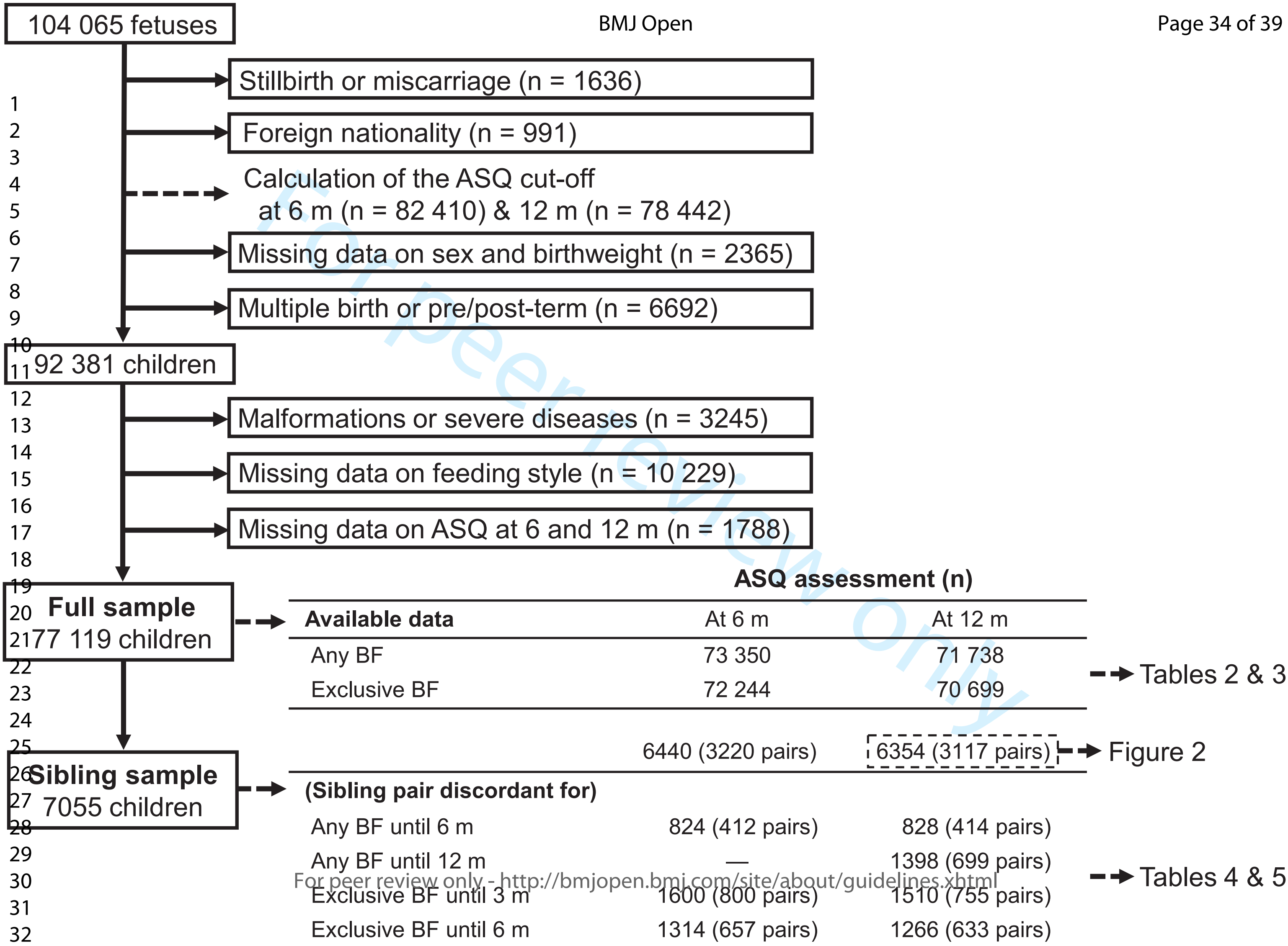
[a] Adjusted for sex, gestational age, birthweight, sibling order, maternal smoking and alcohol, and home speech stimulation at 1 month. [b] Adjusted further for the introduction of complementary food. Boldface represents statistical significance ($P < 0.05$).
Abbreviations: ASQ, Ages and Stages Questionnaires; BF, breastfeeding; CI, confidence interval

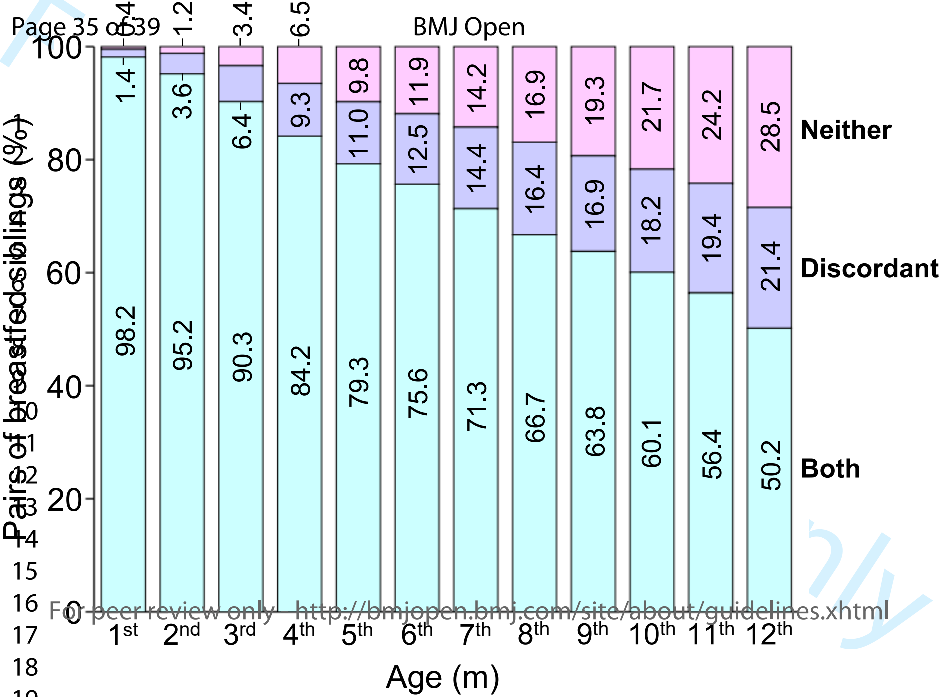
Figure Legends

Figure 1. Flowchart of participant selection.

ASQ, Ages and Stages Questionnaires; BF, breastfeeding

Figure 2. Pairs of siblings who were both breastfed, discordantly breastfed, or neither breastfed with respect to each month of life (n = 3117).


104 065 fetuses
Stillbirth or miscarriage (n = 1636)
Foreign nationality (n = 991)
Calculation of the ASQ cut-off at 6 m (n = 82 410) & 12 m (n = 78 442)
Missing data on sex and birthweight (n = 2365)
Multiple birth or pre/post-term (n = 6692)
92 381 children
Malformations or severe diseases (n = 3245)
Missing data on feeding style (n = 10 229)
Missing data on ASQ at 6 and 12 m (n = 1788)
Full sample
77 119 children
Sibling sample
7055 children
ASQ assessment (n)
Available data
At 6 m
At 12 m
Any BF
73 350
71 738
Exclusive BF
72 244
70 699
Tables 2 & 3
6440 (3220 pairs)
6354 (3117 pairs)
Figure 2
(Sibling pair discordant for)
Any BF until 6 m
824 (412 pairs)
828 (414 pairs)
Any BF until 12 m
—
1398 (699 pairs)
Exclusive BF until 3 m
1600 (800 pairs)
1510 (755 pairs)
Exclusive BF until 6 m
1314 (657 pairs)
1266 (633 pairs)
Tables 4 & 5


0.4
1.2
3.4
6.5
100
80
60
40
20
0
Pairs of breastfed siblings (%)
1.4
98.2
3.6
95.2
6.4
90.3
9.3
84.2
9.8
11.0
79.3
11.9
12.5
75.6
14.2
14.4
71.3
16.9
16.4
66.7
19.3
16.9
63.8
21.7
18.2
60.1
24.2
19.4
56.4
28.5
21.4
50.2
Neither
Discordant
Both
1st 2nd 3rd 4th 5th 6th 7th 8th 9th 10th 11th 12th
Age (m)

Supplementary Information on

Breastfeeding and infant development in a cohort with sibling pair analysis: the Japan Environment and Children's Study

Masafumi Sanefuji, Ayako Senju, Masayuki Shimono, Masanobu Ogawa, Yuri Sonoda, Michiko Torio, Yuko Ichimiya, Reiko Suga, Yasunari Sakai, Satoshi Honjo, Koichi Kusuhara, Shouichi Ohga, Japan Environment and Children's Study Group

Supplementary Figures

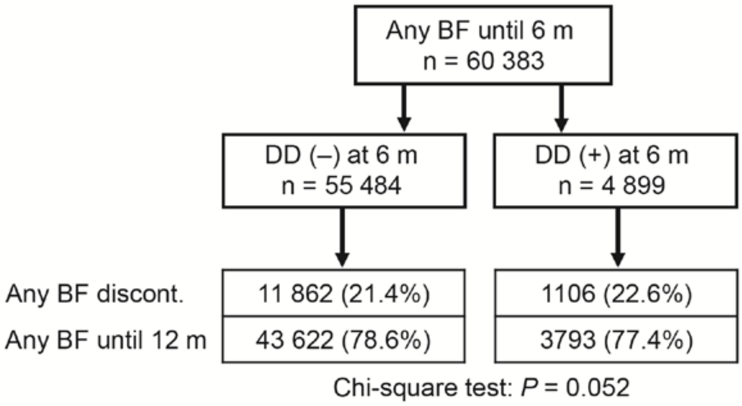


Figure S1. Continuation of any breastfeeding until 12 months in children with *vs.* without developmental delay at 6 months.
BF, breastfeeding; discont., discontinuation; DD, developmental delay

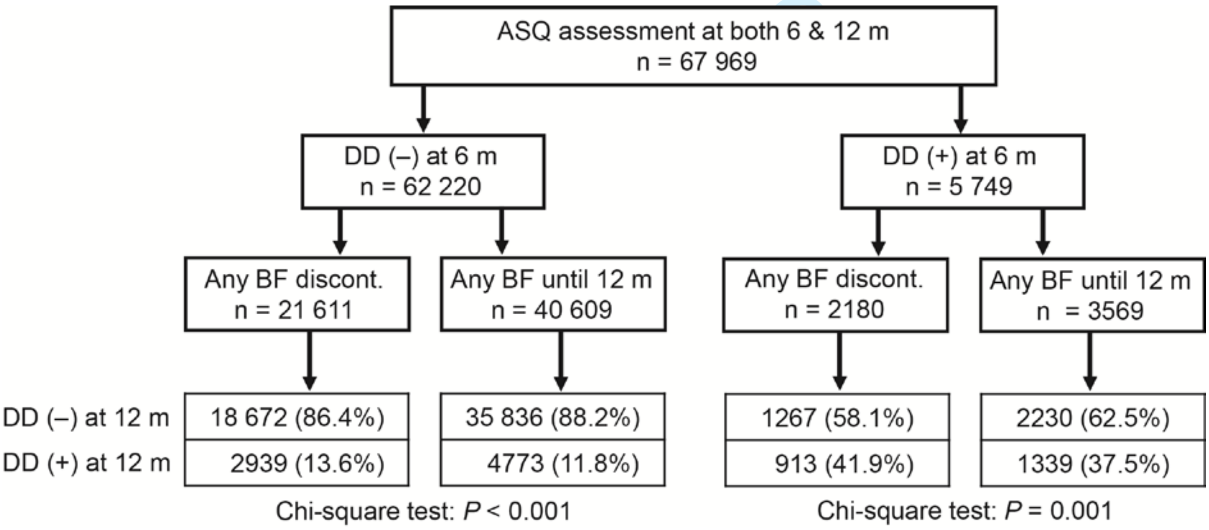


Figure S2. Developmental prognosis in children who continued any breastfeeding until 12 months *vs.* discontinued.
ASQ, Ages and Stages Questionnaires; BF, breastfeeding; discont., discontinuation;
DD, developmental delay.

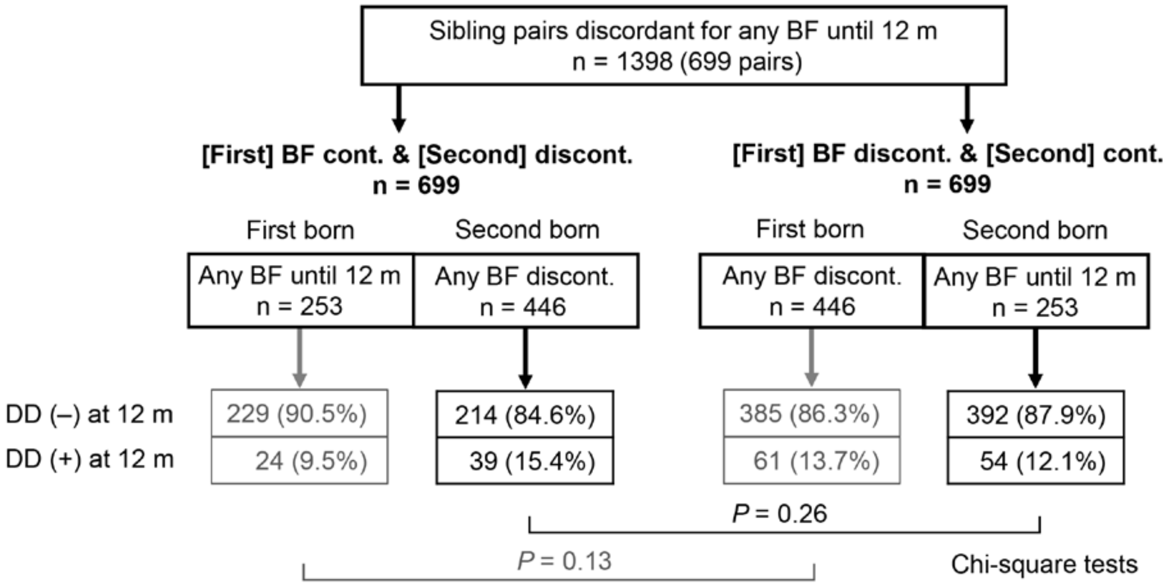


Figure S3. The risk of developmental delay of the second born sibling when the first born sibling continued any breastfeeding until 12 months *vs.* discontinued.

BF, breastfeeding; cont., continuation; discont., discontinuation; DD, developmental delay

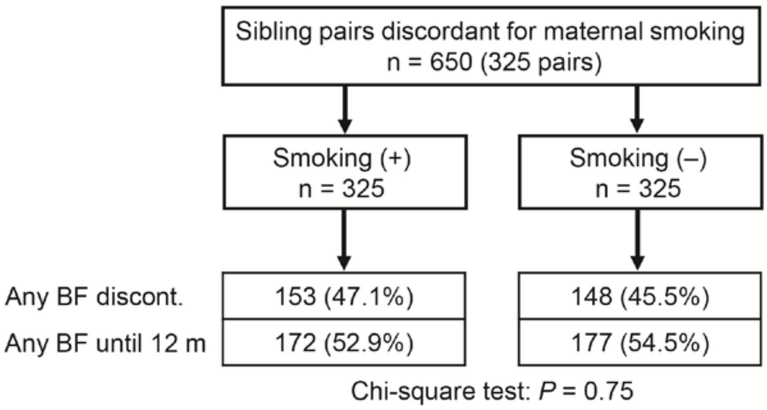


Figure S4. Continuation of any breastfeeding until 12 months in siblings whose mothers smoked during pregnancy *vs.* not.

BF, breastfeeding; discont., discontinuation

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract Confirmed (page 3 of 30)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Confirmed (page 3)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Confirmed (pages 6 & 7)
Objectives	3	State specific objectives, including any prespecified hypotheses Confirmed (page 7)
Methods		
Study design	4	Present key elements of study design early in the paper Confirmed (page 7)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Confirmed (pages 7 & 8)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Confirmed (page 8)
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed Confirmed (page 11)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Confirmed (pages 9-11)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Confirmed (pages 8)
Bias	9	Describe any efforts to address potential sources of bias Confirmed (page 12, Table 1)
Study size	10	Explain how the study size was arrived at Confirmed (page 8)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Confirmed (pages 9 & 10)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding Confirmed (pages 11 & 12)
		(*b*) Describe any methods used to examine subgroups and interactions Confirmed (page 11)
		(*c*) Explain how missing data were addressed Confirmed (page 8, Figure 1, Table 1)
		(*d*) If applicable, explain how loss to follow-up was addressed Confirmed (page 8, Figure 1)
		(*e*) Describe any sensitivity analyses Not applicable

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Confirmed (pages 8 & 12, Figures 1-3, Table 1)
		(b) Give reasons for non-participation at each stage Confirmed (pages 13 & 14)
		(c) Consider use of a flow diagram Confirmed (Figure 1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Confirmed (Table 1)
		(b) Indicate number of participants with missing data for each variable of interest Confirmed (Table 1)
		(c) Summarise follow-up time (eg, average and total amount) Confirmed (page 8)
Outcome data	15*	Report numbers of outcome events or summary measures over time Confirmed (page 12, Table 1)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Confirmed (pages 13 & 14, Tables 2 & 3)
		(*b*) Report category boundaries when continuous variables were categorized Confirmed (pages 13)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Confirmed (pages 13 & 14)
Discussion		
Key results	18	Summarise key results with reference to study objectives Confirmed (page 14)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Confirmed (pages 17)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Confirmed (pages 15 & 16)
Generalisability	21	Discuss the generalisability (external validity) of the study results Confirmed (page 15)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based Confirmed (page 18)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.